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ON A NEW GUM-FORMING STREPTOCOCCUS, WITH STUDIES ON THE OPTIMAL CONDITIONS FOR THE SYNTHESIS OF THE GUM AND ITS PRODUCTION IN COCONUT WATER *

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SIX PLATES

In the course of their study of the microbial flora of "sour mustard," [1] the authors noted that a few of the colonies that developed in plates of tomato-peptone-sucrose agar [2] appeared dry, hard and crystal-like. Crushing a number of these colonies into finer particles in test tubes of tomato-peptone-sucrose solution [3] with slightly acidic reaction and then setting aside the mixture at room temperature, they observed that the liquid

* Received for publication October, 1954.

[1] "Sour mustard" or "borong mostasa," an appetitive food which is relished by many Filipinos, is a product of natural fermentation caused by the action of various types of undetermined microbes on salted mustard [*Brassica integrifolia* (West) O. E. Shutz.] leaves or salted mixture of mustard leaves and rice gruel.

[2] Tomato-peptone-sucrose agar:

Tomato decoction from 250 grams of ripe tomatoes	500.0 cc
Peptone	5.0 gm
Sucrose (refined sugar, with sucrose content of 99.5 per cent)	100.0 gm
$MgSO_4.7H_2O$	0.2 gm
K_2HPO_4	1.0 gm
NaCl	0.1 gm
Agar	20.0 gm
Distilled water to make	1,000.0 cc

[3] Tomato-peptone-sucrose solution is prepared by excluding the agar from tomato-peptone-sucrose agar.

turned turbid after the first day, mucoid after the second day, and gelatinous after the third day. The organism which was responsible for the transformation of the solution into rigid gel appeared to be a coccoid bacterium (Plate 4, fig. 1) as evidenced by dyeing smears prepared from a 12-hour old culture with an ordinary bacterial stain. The solid gel formed by the organism was also found by test to be composed mainly of a polysaccharide of the nature of gum. By dehydrating finer pieces of the gelatinous substance with 95 per cent ethyl alcohol and then drying these in an oven at 100° C, a white amorphous or granular product was obtained.

By hydrolyzing the product in dilute sulfuric acid solution, glucose, among others, was obtained, as evidenced by its being dextro-rotatory and by its positive reaction to Fehling's solution. The relative ease of producing the new substance and the possibility of its being one of economic or industrial importance spurred interest in the following investigation.

THE OPTIMAL CONDITIONS FOR GUM PRODUCTION BY THE NEW UNIDENTIFIED ORGANISM

RELATION OF INITIAL pH OF MEDIUM TO GROWTH AND GUM FORMATION

An experiment to determine the relation of the pH to gum formation by the organism in question was carried in tomato-peptone-sucrose solutions of different pH levels. Each solution was adjusted to the desired pH with the aid of Beckman Model H–2 pH meter and distributed in volumes of 20 cc into sterile 8 × 1-inch culture tubes. The tubes containing the media were autoclaved at 10 pounds pressure for 30 minutes. After cooling them down to room temperature each tube was inoculated with three loopfuls of the water suspension prepared from young cultures of the organism on tomato-peptone-sucrose agar. The cultures were incubated at room temperatures (28° to 32° C) for six days during which they were observed for growth and gum formation. After the sixth day, the batches which showed visible evidences of growth and gum formation were separated and their products were determined quantitatively. This was done by pouring 40 cc 95 per cent ethyl alcohol into each culture tube containing the gum which was previously beaten into finer pieces with one end of a glass rod; leaving the mixture to stand for about 24 hours; filtering

it through a filter paper of known weight; washing the residue with 95 per cent ethyl alcohol; and drying the white gummy substance to constant weight in the oven at 100° C.

Table 1 shows the wide range of initial pH of from 4.0 to 9.0 that the organism could tolerate, the optimal range of from pH 7.0 to 8.5 taken on the basis of firm gel formation, the duration of formation, and the gum yield. The optimal range lies on the alkaline side and may be extended to pH 9.0 if not for the fact that at this pH level the growth of the organism is so slow that gelling becomes evident only after five or six days, instead of the usual three days. For this reason, the pH 9.0 level is not considered as within the optimal range.

TABLE 1.—*Relation of pH to growth and gum production.*

Initial pH of solution	Observations on growth and gum production	Average weight of dry gum produced
		Grams
3.0	No growth; no gel formation	0.0000
4.0	Fair growth; no gel formation	0.0560
5.0	Moderate growth; no gel formation	0.2840
6.0	Abundant growth; with formation of soft gel in 3 days	0.3593
6.5	Abundant growth; with formation of soft gel in 3 days	0.3930
7.0	Abundant growth; with formation of rigid gel in 3 days	0.4933
7.5	Moderate growth; with formation of rigid gel in 3 days	0.4980
8.0	Moderate growth; with formation of rigid gel in 3 days	0.5177
8.5	Moderate growth; with formation of rigid gel in 3 days	0.5377
9.0	Slow growth; with formation of rigid gel after 5 days	0.5230
9.5	No growth; no gel formation	0.0000

The formation of firm gel is not always influenced by profuse growth, as shown in Table 1. At pH 6.0 and 6.5, the growth of the organism is abundant, as shown by the intense cloudiness of the medium during the second day of incubation, but firm gel do not form at these pH levels. On the other hand, at pH 9.0 the growth is very light and hardly perceptible even after three days. The solution, however, is transformed into a firm gel after five or six days. These observations seem to point out that the pH optimum for growth is not the same as that for gelling, the former being slightly on the acid side and the latter, on the alkaline side.

INFLUENCE OF TEMPERATURE ON GROWTH AND GUM PRODUCTION

To determine the most favorable temperature at which the organism could be induced to produce the best development of gum, an experiment was conducted with a tomato-peptone-sucrose solution having an initial pH of 8.0. This medium

was distributed in volumes of 20 cc in fifteen 8 × 1-inch culture tubes. The tubes containing the media were autoclaved at 10 pounds pressure for 30 minutes. After cooling them down to room temperature, each tube was inoculated with 3 loopfuls of the water suspension of a 2-day old culture of the organism on tomato-peptone-sucrose agar. The inoculated tubes were divided into 5 batches of three tubes each and then incubated at temperatures of 25°, 28° to 32° (room temperatures), 37°, 40° and 45° C. After four days the gum produced in each test tube was dehydrated with 40 cc 95-per cent ethyl alcohol and then dried in the oven at 100° C to constant weight

Table 2 shows that the organism develops at temperatures between 25° and 40° C. The temperatures most favorable for gum production appear to be room temperatures ranging from

TABLE 2.—*Relation of temperature to growth and gum production in tomato-peptone-sucrose solution with an initial pH of 8.0.*

Incubation temperature	Observations on growth and gum formation	Average weight of gum produced
Degree centigrade		*Grams*
25	No perceptible growth after the first day; distinctly cloudy after the second day; mucoid after the third day; solid gel after the fourth day.	0.5361
28-32 (Room temp ature)	Distinctly cloudy after the first day; Mucoid after the second day; solid gel on the third day.	0.6656
37	Best initial growth followed by formation of soft gel after the third day.	0.1489
40	Moderate initial growth; no gel formation	0.1120
45	No growth; no gel formation	0.000

28° to 32° C, as shown by the early formation of rigid gel and by the highest yield of gum obtained. The best initial growth of the organism is at 37° C, but this temperature seems to have a repressive effect on the transformation of the medium into solid gel. At 25° C, the development of the organism is somewhat retarded, as shown by the absence of perceptible growth after the first day of incubation and by the delayed gelling of the medium. No growth is observed at 45° C.

The inhibitory effect of low temperatures upon growth has not been definitely determined in this investigation owing to the unavailability of an incubator which could be controlled and maintained at low temperatures. However, in a trial in which three culture tubes containing tomato-peptone-sucrose solution were inoculated with the organism and kept for more

than a week in the lower chamber of a refrigerator wherein the temperatures fluctuated between 11° and 13° C, it was observed that the organism did not grow, thus showing that low temperatures have an inhibitory effect on it.

RELATION OF SUGAR CONCENTRATIONS TO GROWTH AND PRODUCTION OF THE GELATINOUS SUBSTANCE

The foregoing results show that the organism under investigation is capable of producing a gelatinous substance in an alkalinized tomato-peptone-sucrose solution, with sucrose content of 10.0 per cent, when the culture is incubated at room temperatures (28° to 32° C). It is not known, however, whether the organism can be induced to synthesize the gelatinous substance in the same basic medium if the sucrose content were altered and its concentrations varied.

The following experiment is an attempt to clarify this point.

Various concentrations of sucrose (Table 3) in tomato-peptone solutions[4] with neutral reaction to phenolphthalein were prepared in an Erlenmeyer flask using white cane sugar with sucrose content of 99.5 per cent. Each solution was distributed in volumes of 20 cc into three 8 × 1-inch culture tubes and then autoclaved for 30 minutes at 10 pounds pressure. After cooling the tubes down to room temperature, each tube was inoculated with 3 loopfuls of the water suspension from a 2-day old culture of the organism on tomato-peptone-sucrose agar. The cultures were incubated at room temperature for 10 days during which they were examined daily for growth and formation of the gelatinous product.

After 24 hours of incubation, the growth of the organism was perceptible in the unsugared solutions and in all concentrations of sugared solutions up to 40 per cent. In concentrations of 45 to 50 per cent the growth became visible only after four to nine days. In all solutions into which sucrose was added gelling took place, but the formation of firm, solid gel (Plate 2, fig. 2) within a short period of three days occurred only in concentrations of between 8 and 25 per cent (Table 3). In low concentrations, such as in 2 and 4 per cent, an abnormal development of the gelatinous substance was observed, the product being soft and water-soaked. A semisolid gel was formed in the solution with sucrose content of 6 per cent.

[4] Same as tomato-peptone-sucrose solution minus the sucrose.

As shown in Table 3, the growth of the organism gradually slows down until it almost ceases to show up as sucrose concentrations are increased from 30 to 50 per cent. A retarded

TABLE 3.—*Effect of various concentrations of sucrose on the formation of the gelatinous substance.*

Sucrose concentration	Observation on gel formation
Per cent	
0.0	Growth noted but no gel formation was observed.
2.0	Very soft gel after 3 days; gel, watersoaked.
4.0	Soft gel after 3 days; gel, watersoaked.
6.0	Semisolid gel after 3 days with thin layer of clear fluid on top of gelatinous formation.
8.0	Firm gel after 3 days; with thin layer of clear fluid on top of gelatinuos formation.
10.0	Firm gel after 3 days.
12.0	do
16.0	do
18.0	do
20.0	do
25.0	do
30.0	Jellylike after 3 days firm gel after 5 days.
35.0	Jellylike after 3 days firm gel after 12 days.
40.0	Jellylike after 4 days; Not transformed into rigid gel even for a month.
45.0	Growth observed after 4 days; mucoid after 6 days; Jellylike after 2 weeks; Not transformed into rigid gel even for a month.
50.0	Growth observed after 9 days; mucoid after 11 days; not transformed into firm gel even for a month.

development of the organism is observed in solutions containing high sucrose concentrations. In such high sucrose concentrations, the phase of adjustment of the organism to the unfavorable environment is prolonged, and thus its growth takes considerable time before becoming visible.

In the critical concentration of 50 per cent, it is believed that very few microorganisms could thrive. The inhibitory effect of such concentration is such that it prevents the microorganism to grow and to manifest itself within nine days. As soon as signs of growth showed after nine days, platings were immediately made on tomato-peptone-sucrose agar. The development of the characteristic crystal-like colonies in the plates is definite proof that the growth described aboved is produced by the same organism under study.

The optimum development of the gelatinous product in the tomato-peptone-sucrose solutions, as indicated above, ranges from 8 to 25 per cent of sucrose concentrations. A 10 per cent concentration would probably be the most economical to use for a large-scale production of this gelatinous substance.

SOURCES OF NITROGEN THAT PROMOTE GROWTH OF THE ORGANISM AND INDUCE IT TO PRODUCE THE GELATINOUS SUBSTANCE

The present inadequacy of information on the basic or essential nutritional requirements of bacteria has caused microbiologists to use frequently mixtures of organic compounds of unknown chemical composition as culture media. The tomato-peptone-sucrose medium used in the present investigation was a complex mixture of organic substances which, in agar or aqueous form and with appropriate pH and incubation temperature, favored the excellent development of the organism under investigation and influenced it to manifest its most important distinguishing characteristics. Because of the complexity of the chemical composition of the tomato juice in this medium, it may not be possible to determine definitely the essential components in it that stimulate the activity of the organism. Since the gelatinous substance, the desired end-product, consists mainly of a polysaccharide or, may be, a mixture of polysaccharides, its principal source, therefore, must be undoubtedly the sucrose which is supplied to the medium. In the absence of a nitrogenous substance containing a sufficient amount of assimilable nitrogen, an important constituent of cellular protoplasm, the organism would not grow and produce the desired product even if the proper carbohydrate is supplied. In the presence of peptone in the tomato-peptone-sucrose medium the organism shows an excellent performance. Would the same response be shown by the organism if other forms of nitrogenous compounds are used? To answer this question the following experiment to find out in what forms these compounds should be supplied was conducted

Various media such as those enumerated in Table 4, were prepared. In all of them the tomato juice, an important component of the tomato-peptone-sucrose medium, was omitted. Each medium was made neutral to phenolphthalein and distributed in volumes of 20 cc into three 8 × 1-inch culture tubes. The tubes were autoclaved at 10 pounds pressure for one-half hour. After they have cooled down to room temperature, they were inoculated with a water suspension from a 2-day old culture of the organism on tomato-peptone-sucrose agar, and then incubated at room temperatures (28° to 32° C) for 4 days after which the gum yields were determined quantitatively.

As shown in Table 4, the nitrogenous compounds used in this experiment were either of organic or inorganic origin.

TABLE 4.—*Nitrogen sources which influence growth and stimulate gel formation.*

Media	Observations on growth and gum formation	Average weight of gum produced
		Grams
Plain sucrose solution (Sucrose, 10 per cent)	No growth; no gel formation	0.0000
Sucrose salts solution[a]	No growth; no gel formation	0.0000
Peptone, 0.5 per cent + sucrose salts solution	Abundant growth; with formation of very soft gel	0.1049
Yeast extract 0.25 per cent + sucrose salts solution	Abundant growth; with formation of semisolid gel	0.1295
Peptone, 0.5 per cent + yeast extract, 0.25 per cent +: sucrose salts solution	Abundant growth; with formation of semisolid gel	0.1641
$(NH_4)_2SO_4$, 0.1 per cent + sucrose salts solution	No growth; no gel formation	0.0000
$(NH_4)_2SO_4$, 0.1 per cent + peptone, 0.5 per cent + sucrose salts solution	Abundant growth; with formation of soft gel	0.1015
$(NH_4)_2SO_4$, 0.1 per cent + yeast extract, 0.25 per cent + sucrose salts solution	Abundant growth; with formation of semisolid gel	0.1627
$(NH_4)H_2PO_4$, 0.1 per cent + sucrose salts solution	No growth; no gel formation	0.0000
$(NH_4)H_2PO_4$, 0.1 per cent + peptone, 0.5 per cent + sucrose salts solution	Abundant growth; with formation of soft gel	0.0914
$(NH_4)H_2PO_4$, 0.1 per cent + yeast extract, 0.25 per cent + sucrose salts solution	Abundant growth; with formation of semisolid gel	0.2150
KNO_3, 0.1 per cent + sucrose salts solution	No growth; no gel formation	0.0000
KNO_3, 0.1 per cent + peptone, 0.5 per cent + sucrose salts solution	Abundant growth; with formation of soft gel	0.1183
KNO_3, 0.1 per cent + yeast extract, 0.25 per cent + sucrose salts solution	Abundant growth; with formation of semisolid gel	0.1517
$NaNO_3$, 0.1 per cent + sucrose salts solution	No growth; no gel formation	0.0000
$NaNO_3$, 0.1 per cent + peptone, 0.5 per cent + sucrose salts solution	Abundant growth; with formation of soft gel	0.1185
$NaNO_3$, 0.1 per cent + yeast extract, 0.25 per cent + sucrose salts solution	Abundant growth; with formation of semisolid gel	0.1624

[a] Sucrose salts solution: Aqueous solution containing sucrose, 10.0 per cent; $MgSO_4 \cdot 7H_2O$, 0.02 per cent; K_2HPO_4, 0.1 per cent; NaCl, 0.01 per cent.

The organic sources were represented by peptone and yeast extract and the inorganic sources by $(NH_4)_2SO_4$, $NH_4H_2PO_4$, KNO_3 and $NaNO_3$. They were supplied to the medium either singly or in combination of two forms: organic and inorganic. Two other non-nitrogenous media, the first containing sucrose alone and the second, sucrose and salts, were also included for comparison with the nitrogenous media.

Table 4 discloses that the growth of the organism and the formation of the gelatinous substance take place only in the media containing peptone and yeast extract, with these substances serving either as the sole source of nitrogen or in combination with an inorganic form. From these results it may be deduced that, since no development was shown in the media containing $(NH_4)_2SO_4$, $NH_4H_2PO_4$, KNO_3 or $NaNO_3$, the

organism could not use them as the source of food and therefore, could not synthesize new protoplasm from the materials contained in the media.

It appears from Table 4 that yeast extract is a better material to use than peptone as a nitrogenous constituent of the culture medium for the propagation of the organism. This is indicated by the better development of the gelatinous substance and the higher yields of gum in the media containing yeast extract than in those containing peptone. The enhanced development of the organism in the media with yeast extract is believed to be due not solely to the nitrogen contained in the yeast preparation, but also perhaps to the vitamins that the media contained.

The foregoing experiment clearly shows that, as in almost all microorganisms, nitrogen of organic origin is necessary for the development of the organism under study. Even in a medium which is amply supplied with sucrose and mineral salts, the organism could not carry on its normal life processes and produce the gelatinous substance desired if the organic form of nitrogen is omitted.

THE CARBON SOURCES OF THE GELATINOUS SUBSTANCE

As shown elsewhere in the preceding discussion, when the mustard organism is cultivated in an alkalinized medium containing sucrose and organic nitrogenous compound, the medium is transformed into a gelatinous substance within three days, and that when sucrose is omitted or when just a trace of it is present in the medium, the gelatinous product fails to develop. In the experiment (Table 5) in which the influence of sucrose on the production of the gelatinous substance was again tested along with various other carbohydrates, namely arabinose, galactose, glucose, d-mannose, levulose, rhamnose, maltose, lactose, dextrin, raffinose, and a related compound known as mannitol, it was found that the gelatinous product was formed only in the solution which contained sucrose.

The above result seems to point out that sucrose is the only carbohydrate substrate which is suitable for the synthesis of the gelatinous substance. Since this substance is formed only from sucrose and since upon hydrolysis it yields a kind of sugar which is believed to be dextrose, because it reduces Fehling's solution and is dextro-rotatory, the process of its

TABLE 5.—*Showing the results of trials on the use of various carbohydrates and a related compound in concentrations of 10.0 per cent as carbon sources of the gelatinous substance.*

Basal broth	Carbohydrates and related compound added	Relative amount of growth	Observation of gel formation
TOMATO-PEPTONE-SALTS			
Tomato decoction from 250 grams of tomatoes—500.0	Sucrose	Abundant	Rigid gel after 3 days
Peptone—5.0 g	Arabinose	Moderate	No gel formation
$MgSO_4.7H_2O$—0.2 g	Galactose	Abundant	do.
K_2HPO_4—1.0 g	Glucose	do.	do.
NaCl—0.5 g	d-Mannose	do.	do.
Distilled water to make 1000.0 cc	Levulose	do.	do.
pH—8.0	Rhamnose	do.	do.
	Maltose	do.	do.
	Lactose	do.	do.
	Dextrin	do.	do.
	Raffinose	do.	do.
	Mannitol	do.	do.
YEAST EXTRACT-PEPTONE-SALTS			
Bacto yeast extract, Difco—2.5 g	Sucrose	Moderate	Semi-solid gel after 5 days.
Peptone—5.0 g	Arabinose	Slight	No gel formation
$MgSO_4.7H_2O$—0.2 g	Galactose	Moderate	do.
K_2HPO_4—1.0 g	Glucose	do.	do.
NaCl—0.5 g	d-Mannose	Slight	do.
Distilled water to make 1000.0 cc	Levulose	Moderate	do.
pH—8.0	Rhamnose	Slight	do.
	Maltose	Moderate	do.
	Lactose	do.	do.
	Dextrin	do.	do.
	Raffinose	Slight	do.
	Mannitol	do.	do.

development is assumed to be similar to that of "dextran," a polysaccharide which is reported by Baens-Arcega and Yenko (2) to be produced by *Leuconostoc dextranicum* (Beijerinck) Hucker and Pederson from the so-called nascent glucose, a product liberated during the inversion of sucrose.

EFFECT OF LENGTH OF INCUBATION PERIOD ON ACID DEVELOPMENT AND YIELD OF GUM

A 1,000 cc sterile tomato-peptone-sucrose solution was adjusted to pH 7.2. With phenolphthalein as the indicator, the titrable acidity of this solution was found to be equivalent to 0.0042 N. Under aseptic conditions this medium was distributed in volumes of 20 cc into 30 sterile 8 × 1-inch culture tubes each of which was then inoculated with three loopfuls of the water suspension from a 2-day old culture of the organism on tomato-peptone-sucrose agar. The inoculated tubes were placed in a culture chamber at room temperature. After every 24 hours for six consecutive days, five tubes were removed. Two of these tubes were used for pH and titrable acidity deter-

minations and the remaining three, for yield of gum. The data from these determinations are given in Table 6.

The growth of the organism in all the tubes appears to be uniform, as shown by the cloudiness of similar opacity of the solution after 24 hours of incubation. As in previous observations, the formation of the gelatinous substance passes through phases in which the solution turns colloidal after the first day, viscid after the second day, and then solid gel after the third day. When the culture reaches the gel stage there is no further development noted. Instead, there seem to be a retrogression in the development of the product when the time of fermentation is extended. This observation is deduced from the fact that there is a little shrinking of the gelatinous formation in the tubes which were kept for six days, as indicated by the appearance of a layer of clear fluid on top of the gel. At this stage of culture, the gelatinous product turns soft owing perhaps to slight hydrolysis caused by the increase of acidity. It is also possible that such softening is due to the action of an enzyme which might cause slow digestion of the gelatinous product.

Table 6 shows that during the fermentation of tomato-peptone-sucrose solution, the titrable acidity increases while the pH decreases. The major changes in acidity occurs during the

TABLE 6. *Showing changes in hydrogen-ion concentration, titrable acidity and yield of gum during six days the organism was in culture in tomato-peptone-sucrose solution.*

Days of fermentation	Daily changes in reaction of medium		Daily yield
	pH	Titrable acidity, normality	
			Grams
0 (Uninoculated check)	7.20	0.0042	0.0000
1	5.50	0.0104	0.0411
2	4.00	0.0498	0.4145
3	3.85	0.0633	0.4453
4	3.80	0.0753	0.4307
5	3.75	0.0810	0.4385
6	3.78	0.0799	0.3644

first three days of incubation when the pH drops from 7.2 to 3.85 and the titrable acidity rises from 0.0042 to 0.0633 N. The increase of hydrogen-ion concentration nearly parallels the rise in titrable acidity. Within the same period all of the stages which lead to the development of the gelatinous product

are also observed. The gradual development of the gelatinous substance from the colloidal to the rigid-gel phase is accompanied by a gradual rise of acidity. When its maximum development is reached on the third day the concentration of the acid produced by the organism appears to be at about its highest. The changes which are noted from the fourth to the sixth day of fermentation seem so minor as to be of any significance in connection with the formation of the gelatinous substance.

Gum production in tomato-peptone-sucrose solution is observed wherever there is evidence of growth of the organism. Gum is produced as early as the first day of growth, as evidenced by the formation of white, flocculent precipitate when sufficient amount of 95 per cent ethyl alcohol is added to the culture. The production continues and reaches its peak on the third day, as shown in Table 6. This shows that the duration of incubation, which is necessary to effect a major change in acidity, is the same as the period necessary for the transformation of the substrate into rigid gel. From the above discussion it may be deduced that the best duration of fermentation for the production of gum is three days.

GUM PRODUCTION IN COCONUT WATER

The potentiality of coconut water as a microbial medium and as a substrate in industrial fermentation deserves careful investigation because of the abundance of coconuts in the Philippines. It will be one way of boosting the coconut industry, if some use could be found for coconut water which goes to waste in great quantities in the regions where copra is made. As those who have had experience with it know, coconut water, whenever allowed to stand untreated and unprotected from the inroads of microbes, turns sour after several hours, thus showing that the sugars in it ferment into acids. A microscopic examination of the fermented water would invariably reveal the presence of teeming millions of microbial cells that derive their nourishment from the liquid. This shows that coconut water, as microbial medium, can sustain the growth of microbes particularly those that are less fastidious.

Some pertinent data which may be of value to a study of coconut water as microbial medium may be derived from available literature. For instance, the proximate analyses reported by Miller(11) and Peters(13) show that coconut water contains

all of the basic elements which are necessary in microbial nutrition. The figures given by these two authors, if combined, give the range in concentration of the important components of coconut water to be as follows: protein, 0.23 to 0.43 per cent; carbohydrates, 3.68 to 5.00 per cent; fat, 0.356 to 1.5 per cent; ash, 0.64 to 0.8 per cent; calcium, 0.03 per cent; phosphorous, 0.01 to 0.022 per cent. Sampson(15) gave the analysis of the ash from coconut water as follows: lime, 5.55 per cent; magnesia, 6.55 per cent; phosphoric acid (P_2O_5), 3.7 per cent; and potash (K_2O), 31.6 per cent. The sugars in coconut water were isolated and identified by Caray(5) as sucrose, dextrose, and fructose. Miller(11) reported that coconut water is deficient in vitamin A and is also a poor source of vitamin B, but it contains an appreciable amount of vitamin C. Peters(13) enumerated the members of the B-group of vitamins which are present in coconut water and gave the corresponding amount which each cubic centimeter contains as follows: nicotinic acid, 0.64 μg; pantothenic acid, 0.52 μg; biotin, 0.02 μg; riboflavin, 0.01 μg; folic acid, 0.003 μg.

The above review shows that coconut water contains not only the essential components necessary for growth but also the vitamins of the B-group which, although perhaps not present in optimum concentrations, may still possess some growth-stimulating factor for bacteria. From the data presented above a microbiologist can perhaps determine what substance and how much of it should be supplied to coconut water to render it suitable for the nutritional needs of the organism that he may be dealing with.

In the following experiment the main purpose is to find a process by which the mustard organism under study can be induced to produce gum profusely in coconut water. If it can be demonstrated that production of gum in this liquid is economically feasible and if economic or industrial uses of the gummy substance could be found, then the coconut water, which goes to waste at present, would be a good source of additional income for the coconut farmer.

The test on the production of gum in coconut water was conducted as follows: Coconut water from ripe nuts was drawn under aseptic conditions into a sterilized 2-liter flask. From this flask 100 cc was transferred into a 250-cc flask which was then labelled "plain coconut water." Another 100 cc was transferred into a second 250-cc flask and made alkaline by raising

its original pH of 4.9 to 8.0 with a normal solution of NaOH. To the remaining coconut water in the 2-liter flask, white cane sugar with sucrose content of 99.5 per cent, was added to raise the sucrose concentration to about 10.0 per cent. In raising the concentration the sucrose originally present in the coconut water was disregarded as Dunstan(7) and Patel(12) found it to be only about 1.0 or less than 1.00 per cent, which concentrations are considered as not sufficiently high to affect significantly the effect of the precise 10.0 per cent concentration. From this sugared coconut water in the 2-liter flask, 100 cc was poured into a third 250-cc flask which was left without any adjustment of reaction. Another 100 cc was poured into another flask with its reaction adjusted to pH 8.0. The rest of the sugared coconut water was distributed in volumes of 100 cc into seven other flasks. To those flasks the same three inorganic ingredients which were used in the preparation of tomato-peptone-sucrose solution were added either singly or in combination of two or all of them and then each of the resulting solutions was adjusted to pH 8.0 by adding to each a sufficient quantity of a normal solution of NaOH. When all the eleven flasks containing variously treated coconut water were prepared the content of each flask was distributed in volumes of 20 cc into three 8 × 1-inch culture tubes. All the tubes were plugged and then steamed for 30 minutes at 100° C everyday for three consecutive days. After cooling them down to room temperature each tube was inoculated with the water suspension from a 2-day old culture of the bacteria on tomato-peptone-sucrose agar. The cultures were incubated at room temperatures (28° to 32° C). The gum harvests were partially dehydrated in alcohol and then completely dried in the oven at 100° C.

As shown in Table 7, there is an abundant growth of the organism in all solutions after 24 hours of incubation at room temperatures. During this early stage of growth no perceptible difference between any of the treatments is noticed as indicated by cloudiness of approximately the same intensity of the solutions in all tubes. But growth and gum production do not appear to be directly related, otherwise, more or less similar formation of the gelatinous substance should develop in the different solutions. On the third day of growth gelling takes place but this is observed only in alkalinized solutions containing cane sugar. Whether such solutions contained the organic ingredients or not, similar rigid formation of the gela-

tinous substance develop in them. No gelatinous formation is observed in the plain coconut water, in alkalinized coconut water without sugar, and also in the tube to which sugar was added but not subsequently alkalinized.

TABLE 7.—*Gum production in coconut water.*

Media		Observations on growth and gum formation		Average weight of gum produced
Composition	pH	Relative amount of growth	Characteristics of gum formation	
				Grams
Plain coconut water	4.9[b]	Abundant, milky hue	No gel formation	0.0667
Plain coconut water	8.0[c]	do	do	0.0951
Coconut water + 10.0 per cent cane sugar	4.9	do	do	0.0877
Coconut water + 10.0 per cent cane sugar	8.0	do	Gel formation, rigid after 3 days.	0.8388
Coconut water + 10.0 per cent cane sugar + 0.02 per cent $MgSO_4.7H_2O$	8.0	do	do	0.8577
Coconut water + 10.0 per cent cane sugar + 0.10 per cent K_2HPO_4	8.0	do	do	0.8420
Coconut water + 10 per cent cane sugar + 0.05 per cent NaCl	8.0	do	do	0.8230
Coconut water + 10.0 per cent cane sugar + 0.02 per cent $MgSO_4.7H_2O$ + 0.1 per cent K_2HPO_4	8.0	do	do	0.8473
Coconut water + 10.0 per cent cane sugar + 0.02 per cent $MgSO_4.7H_2O$ + 0.05 per cent NaCl	8.0	do	do	0.8401
Coconut water + 10 per cent cane sugar + 0.1 per cent K_2HPO_4 + 0.05 per cent NaCl	8.0	do	do	0.8318
Coconut water + 10 per cent cane sugar + 0.02 per cent $MgSO_4.7H_2O$ + 0.1 per cent K_2HPO_4 + 0.05 NaCl	8.0	do	do	0.8369

[b] pH 4.9 (original pH of coconut water).
[c] pH 8.0 (adjusted).

While coconut water permits a profuse growth of the organism, it is an unsuitable medium for the production of the gelatinous substance because its sucrose content and pH is not within the optimum limits required by the organism for the production of this substance. It is seen in Table 7 that by making the coconut water alkaline and by raising its sucrose concentration to about 10.0 per cent, a condition favorable for the production of gum is established. It also appears from a close inspection of the gum yields from different treatments (Table 7) that the addition of the inorganic salts gives no distinct beneficial effect to warrant their use as supplementary ingredients designed to replinish the seemingly low salt content of coconut water. This result shows that the salts originally present in coconut water must occur in amounts sufficient to sustain the needs of the organism. Samples of the dried

products that were synthesized by the organism in coconut water are shown in Plate 1, fig. 1.

CULTURAL CHARACTERISTICS

Agar colonies.—On tomato-peptone-sucrose agar with sucrose content of about 10 per cent and pH of between 7 and 8, the growth of the mustard organism at 28° to 32° C is so typical that it could easily be distinguished from that of any other bacterium. On this medium the colonies become visible usually after 36 hours. Young colonies, whether surface or submerged, are opaque, granular, and either circular or ellipsoidal. After three days, the colonies become white, nodular, with rugged surface and irregular margin, lustrous, hard and crystal-like in appearance. Some of the submerged colonies may remain opaque sometime longer, but later they also become lustrous, nodular and crystal-like in appearance. In a plate where fully developed colonies are not so thickly distributed as to exceed 400 in number, they appear as crystal lumps on the agar (Plate 3, fig. 1). They usually measure between two and three millimeters but they may reach five or more millimeters if only a few of them are growing in the plate. As the colonies grow and develop they seem, in some cases noted, to eat up the agar immediately below them, thus appearing as if they are sunk in the agar (Plate 3, fig. 2). As a consequence of this disturbance, cracks appear in the agar. The cracks originate from the margin of the eaten-up area on the agar and elongate as the agar dries out (Plate 3, fig. 3).

On beef-extract agar the colonies are usually punctiform, the surface smooth, the edge entire or slightly undulate, and the body, amorphous. Larger colonies are produced when the initial reaction of this medium is made slightly alkaline.

On potato-dextrose agar the colonies are about the same in size as those on beef extract agar. They are punctiform, the surface rough, the edge undulate. Colonies as large as 1.5 millimeters are produced when this medium is made slightly alkaline.

On Sabouraud's agar the colonies show the same characteristics as those produced on potato-dextrose agar.

Agar slant.—On tomato-peptone-sucrose agar, the growth is abundant, white, crystal-like, and usually beaded, but may become almost filiform as the colonies coalesce. The surface is rough, at first glistening, but may become dull as the culture

ages. In very old cultures the crystal-like growth (Plate 1, fig. 2) may at certain points along the line of inoculation slough off from its contact with the surface of the agar.

On an agar which contains dextrose, such as tomato-peptone-dextrose, potato-dextrose, beef extract-dextrose, Sabouraud's and bacto tryptone-glucose-extract-milk agar, the growth of the organism is moderate, beaded with smooth glistening surface and with butyrous consistency. With age the growth loses somewhat its luster.

Agar stab.—In an agar containing dextrose the growth in stab is moderate, uniform from top to bottom, filiform or beaded along the line of inoculation.

In tomato-peptone-sucrose agar the growth is abundant, white, uniform from top to bottom, usually beaded. The colonies are nodular and crystal-like.

Growth in broth.—The standard meat extract broth becomes scantily clouded in 48 hours at room temperature. The cloudiness gradually settles down as fine, white sediment. No surface growth is formed.

The tomato-peptone-sucrose solution with either 1 or 10 per cent and with pH of 5 or less becomes moderately clouded in 24 hours at room temperature. A white membranous or granular growth is sometimes observed at the bottom of the culture tubes. The organism quickly and densely clouds the same medium made slightly alkaline with normal solution of NaOH. The cloudiness gradually settles down either in the form of white flocculent sediment of tangled chains of cells (Plate 4, fig. 3) or as loose soft mass of gelatinous substance whenever the sucrose content of such medium is only 1 per cent. But if the clouded solution contains 10 per cent sucrose it is transformed into rigid gel in 3 days.

The tomato-peptone-dextrose becomes densely clouded in 24 hours at room temperature. The cloudiness gradually settles down as fine white sediment. No formation of the gelatinous substance occurs in this medium even if its reaction is made alkaline with NaOH.

MORPHOLOGICAL CHARACTERISTICS

Form and size of cells.—The mustard organism is a chain-forming coccus which measures from 0.5 to 0.8 micron in diameter. Freshly isolated from fermenting mustard, this organism appears in the form of short chains or diplos (Plate 4, fig. 1).

After keeping the organism in artificial culture for sometime, especially in an enriched agar medium, it becomes variable in form and in certain cases distinctly pleomorphic. The individuals elongate to generally short rods (Plate 6, figs. 1 and 2), which vary from 0.6 to 2.0 microns in length and from 0.4 to 0.6 micron in width. From a 2-day old culture on bacto-tryptone glucose extract milk agar, cells which have grown out into slender rods or short filaments measuring from 10 to 15 or more microns long have been observed (Plate 6, fig. 4). Cultivating the organism in an unfavorable medium such as standard nutrient broth, it becomes again a chain-forming coccus (Plate 4, fig. 2). In a 20-hour old culture in tomato-peptone-sucrose solution, the organism may be seen either in pairs or chains of usually not more than 12 cells. In older cultures, chains of more than 20 units may frequently be observed. From a crystal-like colony which develops on tomato-peptone-sucrose agar, long, crisscross chains of cells, which are embedded in the gummy substance, are always seen (Plate 6, fig. 3).

Motility.—The organism does not manifest any sign of motility in tomato-peptone-sucrose solution or any other broth. A stab culture of the organism in the motility test medium does not show any spreading-out of growth from the line of inoculation.

Staining reactions.—The organism stains readily with ordinary laboratory dyes such as Ziehl's carbol fuchsin, Loeffler's alkaline methylene blue and safranin.

It is gram-positive and not acid-fast. No endospores or flagella are observed.

Capsules (Plate 5, fig. 1) are present and are clearly demonstrated from a 22-hour old culture on tomato-peptone-sucrose solution by means of a combination of simple staining with Ziehl's carbol fuchsin and relief staining with nigrosin solution. Large capsules appear to be formed by the coalescence of several encapsulated chains of cells (Plate 5, fig. 2). The gelatinous substance which has been described previously to have been formed in tomato-peptone-sucrose solution, might have been the result of the fusion of a great many, if not all, of those encapsulated chains of cells.

BIOCHEMICAL REACTIONS

Fermentation of carbohydrates and related compounds.—The fermentation of carbohydrates and related compounds was car-

ried in a sterile basal broth, as mentioned in the heading of Table 8, by adding an equivalent of 0.5 per cent of the test substance. All solutions containing the test substances were sterilized by filtering through sterilized Mandler Bacteriological Filter except those containing starch, inulin and dextrin which were sterilized by steaming without pressure for 30 minutes each day for three consecutive days. Under aseptic conditions each solution was distributed in volumes of 20 cc into graduated Smith fermentation tubes. Each tube was inoculated with 3 loopfuls of the bacterial suspension from a 2-day old culture of the organism under study on tomato-peptone-dextrose agar. Inoculated tubes containing the basic broth but without the test substance, and uninoculated tubes containing both the basic broth and the test substance were maintained as checks.

As shown in Table 8 all the pentoses (arabinose, rhammose, and xylose), hexoses (glucose, fructose, galactose and d-mannose) and disaccharides (lactose, sucrose, maltose, trehalose,

TABLE 8.—*Fermentation of carbohydrates and related compounds in a basal broth consisting of bacto-tryptose 5.0 grams, Difco yeast extract 2.5 grams, NaCl 0.5 gram K_2HPO_4 1.0 gram, distilled water 1,000 cc; reaction adjusted to pH 7.5; bromcresol purple added as indicator.*

Fermentable substance tested	Reaction after 10 days	Gas production
	pH	
Arabinose	5.20	None
Rhamnose	4.80	do.
Xylose	5.10	do.
Glucose	4.20	do.
Fructose	4.00	do.
Galactose	4.65	do.
d-Mannose	4.00	do.
Lactose	4.20	do.
Sucrose	4.00	do.
Maltose	4.20	do.
Trehalose	3.95	do.
Melibiose	4.10	do.
Cellobiose	4.10	do.
Raffinose	4.40	do.
Melizitose	4.40	do.
Starch	6.40	do.
Inulin	6.30	do.
Dextrin	5.65	do.
Glycerol	5.20	do.
Adonitol	No growth	
Mannitol	4.40	None
Sorbitol	5.25	do.
Dulcitol	No growth	
Inositol	No growth	
Aesculin	4.30	None

melibiose and cellobiose) were readily fermented with the production of much acid. Much acid production from the trisaccharides, raffinose and melizitose, is also noted. Clouding in tubes containing starch and inulin is noted, but the acid produced is not sufficient to change the indicator from alkaline

to its acid color. Of the polyhydroxy alcohols included in the test, only glycerol, mannitol and sorbitol are fermented also with the production of acid. Adonitol and dulcitol are unchanged as no sign of growth was observed in tubes containing these substances. The cyclic hexahydric alcohol known as inositol is not fermented. The glucoside aesculin is readily fermented with the production of much acid.

Table 8 also shows that in all the tubes wherein fermentation takes place there is always an acid production, but there is no case in which active gas is evolved. All of the uninoculated controls do not show any change of color. The inoculated check tubes which contained only the basic broth show slight growth but no change in the color of the indicator is observed, thus indicating that the ingredients used in the preparation of the broth did not contain any fermentable substance.

Gelatin liquefaction.—The test on gelatin liquefaction was carried in a modified gelatin medium [5] which was found suitable for the growth of the organism. After the tubes containing the gelatin medium have been inoculated with the organism they were distributed together with the uninoculated controls into two incubation chambers, the first having a temperature of 25° C, the second having 28° to 32° C (room temperatures). These temperatures did not favor gelling of the gelatin medium, but supported the good growth of the organism.

Although growth was noticed after three days, the tubes were kept in the incubation chambers for one week. They were then chilled in a refrigerator to ascertain whether or not there was any liquefaction of gelatin. The growth in the gelled medium was moderate at 25° C and fairly abundant at 28° to 32° C. It was filiform, uniform from top to bottom somewhat warty along the margin. No liquefaction of gelatin was observed.

[5] Medium used for the study of gelatin liquefaction:

Peptone, Bacto	10.0 gm
NaCl	5.0 gm
Dextrose	100.0 gm
Reef extract	3.0 gm
Yeast extract	5.0 gm
Gelatin	120.0 gm
Gater to make	1,000.0 cc

Action on bacto-litmus milk.—Acid production became noticeable after two days when the litmus milk changed its color from blue to pink. Curdling of milk and reduction of litmus occurred simultaneously. In two weeks a large amount of curd was observed and reduction of litmus was almost complete. No peptonization of the curd took place.

Indole production.—The culture used in this test was carried in tryptone broth with reaction adjusted to pH 7.5 in order to get a good growth of the organism. Following the Ehrlick-Bohme technique the test for the presence of indole was done in 24-, 48-, and 72-hour old cultures. Indole was not detected in cultures containing the mustard organism, but it was formed in check tubes containing the cultures of *Escherichia coli* (Migula) Castellani and Chalmer.

Action on nitrates.—By culturing the organism in nitrate broth and checking the presence of nitrite daily for three days by adding 1.0 cc of sulfanilic acid and 1.0 cc of a-naphthylamine reagent solutions, a negative reaction was always obtained. The check tubes containing the nitrate-reducing *E. coli* always gave a distinct red color which indicated the presence of nitrite.

Hydrogen sulfide production tests.—Stab inoculation of the organism under study into tubes of lead acetate agar and keeping the tubes at room temperature for more than a week did not produce any visible blackening along the line of growth, thus indicating that hydrogen sulfide was not produced. Hydrogen sulfide, on the other hand, was formed in check tubes containing cultures of *Salmonella typhosa* (Zopf.) White.

RELATION TO FREE OXYGEN

Test 1 (*Shake culture*) (*6*).—Three test tubes each containing 15 cc of tomato-peptone-sucrose agar were heated in a water bath at boiling temperature and then cooled down to 45° C at which temperature they were inoculated heavily with the young culture of the organism. The inoculated tubes were rotated briskly to insure uniform distribution of the bacterial cells throughout the medium. Then they were allowed to cool down to room temperature. After incubating the tubes for two days, colonies began to form and to be visible throughout the medium from top to bottom. After four days, the colonies became distinctly nodular, with irregular margin, and crystal-

like in appearance. The colonies situated deep in the column of the agar were just as big as those located throughout the upper part, showing that the organism could live even in the absence of air.

Test 2 (*Petri dish culture*) (*6*).—Tubes containing melted tomato-peptone-sucrose agar were heavily inoculated with the young culture of the organism, poured into inverted tops of Petri dishes, and then covered with their respective bottoms hollow sides up, to exclude the air from the top of the agar. The colonies that developed in the agar below the upper glass and also those along its periphery showed practically no difference in form and size, indicating that the organism could thrive under both aerobic and anaerobic conditions.

Test 3 (*Production of gelatinous substance under aerobic and anaerobic conditions*).—Six (150 × 15 mm) culture tubes containing tomato-peptone-sucrose solution were heated in a water bath at boiling temperature to drive off the air from the solution. After having cooled down to 45° C, they were all inoculated heavily with a young culture of the organism. Melted sterile vaseline was poured into three inoculated tubes to a thickness of three-fourths inch. The vaseline was remelted by flaming the parts of the tubes where it was located in order to seal properly the medium from the air. All of the inoculated tubes were incubated at room temperature.

On the third day of incubation the solutions in all tubes were transformed into rigid gel. Gas production was not observed even in the tubes containing the vaseline, verifying further a previous observation that the organism is not a gas-former. Since no marked difference in gel formation was noted between tubes with vaseline and those without it, this test shows that the organism is capable of respiring facilely with or without air.

THERMAL DEATH POINT

The thermal death point of the organism under investigation was determined by the capillary-tube technique (6). Sterile thin-walled capillary tubes, 6 centimeters long and 1 to 1.5 millimeters in internal diameter were partly filled with the young culture of the organism in tomato-peptone-sucrose solution and then the empty ends were sealed over the alcohol flame. In a temperature-controlled warm bath, the cultures were exposed to heat at increasing degrees of 5° C intervals

beginning at 45° C for 10 minutes each. The capillary tubes were broken in the test tubes of melted tomato-peptone-sucrose agar which has been cooled down to 40° C and then the agar was immediately poured into sterile culture plates. The growth of the organism in the plates was easily recognized after two or three days by the crystal-like appearance of the colonies.

From the test described above, it was found that the organism was not very resistant to heat. It was killed at 60° C in 10 minutes, but many cells were still alive after exposing the cultures for the same length of time at 55° C. When the cultures were exposed for 10 and 5 minutes at 57.5° C, no live cells in either case were left to reproduce the crystal-like colonies in the culture plate. This result seems to point out that the heat which the organism can withstand for the given time of 10 minutes lies close to 57.5° C. This figure is within the range of thermal death point (55° to 65° C, for 10 minutes) given by Kelly and Hite(10) for most nonsporulating bacteria.

LACTIC ACID FROM SUCROSE, DEXTROSE, LACTOSE AND MALTOSE FERMENTATION

In the preceding fermentation test in which acid without gas was formed from nearly all the carbohydrates tried, no emission of sour odor was detected, indicating that the acid might be lactic, a nonvolatile acid which sometimes forms as one of the end products of similar fermentations. The following test was designed to determine if this acid was formed by the organism under study from the fermentation of common sugars, namely sucrose, dextrose, lactose and maltose.

The organism was cultivated in several 8 × 1-inch culture tubes each containing 20 cc of 3 per cent solution of the sugars mentioned above in peptone-yeast broth.[6] The tubes were then analyzed qualitatively for lactic acid after the cultures have been incubated for five days at room temperature. The technic of Arny and Dimler(1) was followed in determining the presence of lactic acid. Following this technic, lactic acid was extracted with ethyl acetate and then Brauer's resorcinol test(3) was applied. The analyses were paralleled by a check test using 0.5 cc of 10 per cent lactic acid.

[6] Peptone-yeast-broth: peptone, 5 grams; bacto-yeast-extract, 5.0 grams; $MgSO_4.7H_2O$, 0.2 gram; NaCl, 0.5 gram; K_2HPO_4, 1.0 gram; distilled water, 1,000 cc; pH, 8.0.

The tests, in which the positive results were indicated by the reddish brown color when a mixture of resorcinol solution and the steam-bath-dried ethyl acetate extract of the fermented sugar solutions was layered upon sulfuric acid, revealed conclusively that lactic acid was produced from sucrose, dextrose, lactose and maltose. In these qualitative analyses, the ethyl-acetate-extracted lactic acid always gave a darker color reaction in the presence of resorcinol and sulfuric acid than the check lactic acid, indicating that an appreciable quantity of this compound was extracted from the fermented material.

TAXONOMY

The morphological characteristics and biochemical reactions of the organism under present investigation indicate that it should be classified, in accordance with Bergey's Manual of Determinative Bacteriology(4), as a member of the family Lactobacteriaceæ, a group of lactic acid-forming bacteria. As shown in the Manual, this family is split into two tribes; namely Streptococceæ, the coccoid group, and the Lactobacilleæ, the rod group. By reason of its being a chain-forming coccus, as clearly indicated during its primary isolation, the organism in question should be placed in tribe Streptococceæ under which three genera, namely *Diplococcus, Streptococcus,* and *Leuconostoc,* are recognized. The unknown organism is definitely not a *Diplococcus* because it usually forms longer chains than diplos although in certain cultures the chain sometimes break into adjacent pairs. Besides this, it is definitely gram-positive both in young and old cultures, while members of genus *Diplococcus* show gram-positive reaction only in young culture and a tendency to become gram-negative as the culture ages. This organism may, therefore, be either *Streptococcus* or *Leuconostoc.*

The genera *Streptococcus* and *Leuconostoc* have been considered by Hucker and Pederson(9) to be intermediate between two morphological groups which should be classified as truly spherical like the micrococci, and as true rods like the lactobacilli, because the morphology of these two genera is variable—cocci under certain conditions and rods in certain cultures. The same authors have shown that "the members of genus *Leuconostoc* are intermediate in form between the streptococci and the lactobacilli," indicating that the leuconostocs are more remarkably pleomorphic than the members of genus *Strepto-*

coccus. From the morphological standpoint the new organism under consideration seems to be closer to genus *Leuconostoc* than to genus *Streptococcus* by reason of its being highly pleomorphic. But Bergey's Manual(4) separates the two genera mainly on the basis of glucose fermentation. On this basis the unknown organism should be placed under genus *Streptococcus*, because it forms lactic acid from glucose without the production of active carbon dioxide gas. The leuconostocs, according to the same Manual, produce from glucose carbon dioxide, lactic acid, acetic acid and ethyl alcohol.

There are many species of *Streptococcus* of which only twenty-four are recognized in Bergey's Manual(4), the rest being described or listed as among those with vague or doubtful taxonomic relationship. While it can be indicated that the present unknown organism is, in certain respects, morphologically and physiologically related with certain species described in the Manual, it can also be shown that it possesses other characteristics which are distinctly its own and which have not been attached to any of the described members of genus *Streptococcus*. Of these characteristics, the production by it of crystal-like colonies and gelatinous substance respectively in an enriched sucrose agar and sucrose solution may be considered as outstanding.

A bacterium which is reported by Dyar(8) to produce "clear, refracting, checkered-crystalline" growth on agar and named by him *Bacillus crystalloides* = [*Bacterium crystalloides* (sic) Chester](4) is in no way similar to the organism under study either in morphology or biology, as it is described as an aerobic spore-forming bacillus which quickly liquefies gelatin and could reduce nitrates. The organism in question is, therefore, a distinct species of *Streptococcus* for which the authors propose the binomial *Streptococcus crystalloides* sp. nov., comb. nov.

SUMMARY

1. A new gum-forming coccoid bacterium was isolated from "sour mustard," a native food product which results from natural fermentation of salted mustard leaves. This organism when cultured at ordinary room temperature in an alkalinized tomato-peptone-sucrose solution or coconut water with sucrose content of 10 per cent or more produces a rigid gelatinous substance.

2. This gelatinous substance is believed to be composed mainly of a polysaccharide which, when hydrolyzed with an acid, yields dextrose. This seems to indicate that the new product may be one form of dextran, or one related to it.

3. Studies of the morphological and biochemical characteristics of the coccoid bacterium reveal that it is a distinct species of *Streptococcus* which has not heretofore been reported.

4. The new unknown organism, for which the binomial *Streptococcus crystalloides* sp. nov., comb. nov. is proposed, may be described as follows:

Cells spherical, 0.6 to 0.8 micron, usually occurring in chains or in pairs, non-motile, non-spore-forming, gram-positive, non-acid-fast, capsulated when growing in sucrose solution which has been enriched with tomato or yeast extract; may elongate into usually short rods when grown in a favorable medium or when kept in artificial culture for a long time. Gelatin stab, filiform white growth; no liquefaction. Litmus milk, curdled but not peptonized; litmus reduced simultaneously. Nitrate not reduced. Indole not formed. Hydrogen sulfide not produced. Falcultative anaerobe. Forms acid but no gas from arabinose, rhamnose, xylose, glucose, fructose, galactose, d-mannose, lactose, sucrose, maltose, trehalose, melibiose, cellobiose, raffinose, melizitose, dextrin, glycerol, mannitol, sorbitol and aesculin; little acid from starch and inulin; no growth in adonitol, dulcitol, and inositol. Produces lactic acid from sucrose, dextrose, lactose, and maltose.

Distinctive characters: Produces crystal-like colonies on tomato-peptone-sucrose agar with slightly alkaline reaction. Transforms tomato-peptone-sucrose solution or sugared coconut water with slightly alkaline reaction and with sucrose content of 10 per cent or more into a rigid gelatinous substance.

Optimal conditions for the production of gum: pH, 7.0 to 8.5; temperature, 28° to 32° C (room temperatures); nitrogen source, organic in origin; carbon source, sucrose; sucrose concentration 10 to 25 per cent. Gum development enhanced by certain growth-promoting factors present in tomato extract, coconut water, or yeast extract.

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ILLUSTRATIONS

PLATE 1

FIG. 1. In glass-stoppered bottles are dried products synthesized by *Streptococcus crystalloides* sp. nov. in coconut water which has been sweetened with 10 per cent refined cane sugar; about × 1. Left, from the granular growth produced in slightly acidic coconut water medium. Right, from the gelatinous substance produced in slightly alkaline coconut water medium.

2. Ten-day old tube cultures of *S. crystalloides* on tomato-peptone-sucrose agar showing the crystal-like growth about × 1. Left, line and stab inoculation on slant agar. Right, line inoculation on slant agar.

PLATE 2

FIG. 1. Showing the development of *S. crystalloides* at different pH levels in 8 × 1-inch culture tubes in tomato-peptone-sucrose solutions; about × 0.25. Note the formation of the gelatinous substance from pH 6.0 to pH 8.5. When this was photographed after the fourth day of incubation, the solution with pH of 9.0 did not show any gelling at all but two days later it was transformed into a rigid gel. No growth was shown at pH 3.0 and pH 9.5.

2. Gum production by *S. crystalloides* in 8 × 1-inch culture tubes containing various concentrations of sucrose in tomato-peptone-sucrose solution; about × 0.25.

No. 1. Uninoculated solution.
No. 2 to 12. Inoculated solutions.
2. Without sucrose.
3. With 2.0 per cent sucrose.
4. do 4.0 do
5. do 6.0 do
6. do 8.0 do
7. do 10.0 do
8. do 12.0 do
9. do 14.0 do
10. do 16.0 do
11. do 18.0 do
12. do 20.0 do

Note that the other culture tubes used in this experiment, such as those with concentrations of 25, 30, 35, 40, 45, and 50 per cent, are not included in the photograph.

3. Gum production by *S. crystalloides* in 8 × 1-inch culture tubes containing different kinds of coconut water media; about × 0.25.

No. 1. Uninoculated plain coconut water.
No. 2 to 12. Inoculated coconut water media.
2. Plain coconut water; pH 4.9.
3. Plain coconut water; pH adjusted to 8.0.

4. Coconut water + 10.0 per cent cane sugar; pH 4.9.
5. Coconut water + 10.0 per cent cane sugar; pH adjusted to 8.0.
6. Coconut water + 10.0 per cent came sugar + 0.02 per cent $MgSO_4.7H_2O$; pH adjusted to 8.0.
7. Coconut water + 10.0 per cent cane sugar; + 0.1 per cent K_2HPO_4; pH adjusted to 8.0.
8. Coconut water + 10.0 per cent cane sugar + 0.05 per cent NaCl; pH adjusted to 8.0.
9. Coconut water + 10.0 per cent cane sugar + 0.02 per cent $MgSO_4.\ 7H_2O$ + 0.1 per cent K_2HPO_4; pH adjusted to 8.0.
10. Coconut water + 10.0 per cent cane sugar + 0.02 per cent $MgSO_4.7H_2O$ + 0.05 per cent NaCl; pH adjusted to 8.0.
11. Coconut water + 10.0 per cent cane sugar + 0.1 per cent K_2HPO_4 + 0.05 per cent NaCl; pH adjusted to 8.0.
12. Coconut water + 10.0 per cent cane sugar + 0.02 per cent $MgSO_4.7H_2O$ + 0.1 per cent K_2HPO_4 + 0.05 NaCl; pH adjusted to 8.0.

PLATE 3

Plate culture of *S. crystalloides* on tomato-peptone-sucrose agar.

FIG. 1. A four-day old culture showing the crystal-like growth of the colonies; slightly more than × 1.
2. Enlarged photograph of a ten-day old culture. Note that the crystal-like colonies appear to be sunk in the eaten-up areas on the agar; about × 2.
3. A two-week-old culture showing cracks caused by the rapidly growing colonies in the eaten-up areas on the agar; slightly less than × 1.

PLATE 4

FIG. 1. Photomicrograph of stained cells from a crystal-like growth of a 3-day-old culture of freshly-isolated *S. crystalloides;* approximately × 1,800. Note that the cocoid cells are arranged usually in chains or in pairs. Before staining, the crystal-like growth was first softened or partially dissolved with two or three drops of normal solution of NaOH, washed with several changes of sterile distilled water and then fixed in air at room temperature. This treatment was observed to have caused slight shrinking of the cells.
2. Photomicrograph of stained smear of *S. crystalloides* from a 4-day old culture in standard nutrient broth, a medium in which this organism showed a scanty growth but in which the coccoid form is the predominating type of cells; approximately × 1,800.
3. Photomicrograph of a smear from the flocculent sediment in a 5-day old culture in tomato-peptone-sucrose solution with sucrose content of 1.0 per cent and with pH of 8.0; stained with Ziehl's carbol fuchsin; approximately × 1,800.

PLATE 5

Photomicrograph of capsulated cells of *S. crystalloides* from a 22-hour old culture in tomato-peptone-sucrose solution. Capsules and cells are shown by means of a combination of simple staining with Ziehl's carbol fuchsin and relief staining with nigrosin solution.

FIG. 1. Showing the variation in form of cells—some coccoid, others distinctly rod-shaped; approximately × 1,800.

2. Showing a large capsule (top, left) which appears to have been formed by the fusion of a number of capsulated units or chain; approximately × 1,800.

PLATE 6

FIGS. 1 to 2. Smears from a 48-hour old culture of *S. crystalloides* on beef extract-yeast-dextrose agar showing the short-rod form of cells; approximately × 1,800.

1. Stained with Ziehl's carbol fuchsin.
2. Gram stain; reaction, positive.

3. A thin portion of a young crystal-like colony formed by *S. crystalloides* on tomato-peptone-sucrose showing criss-cross chains of cells embedded in the gummy substance; approximately × 1,800, stained with Ziehl's carbol fuchsin.
4. Stained smear from a 48-hour old culture of *S. crystalloides* on tryptone-glucose-extract milk agar showing cells of various types—coccus, short rod, slender rod, and short filament; approximately × 1,800.

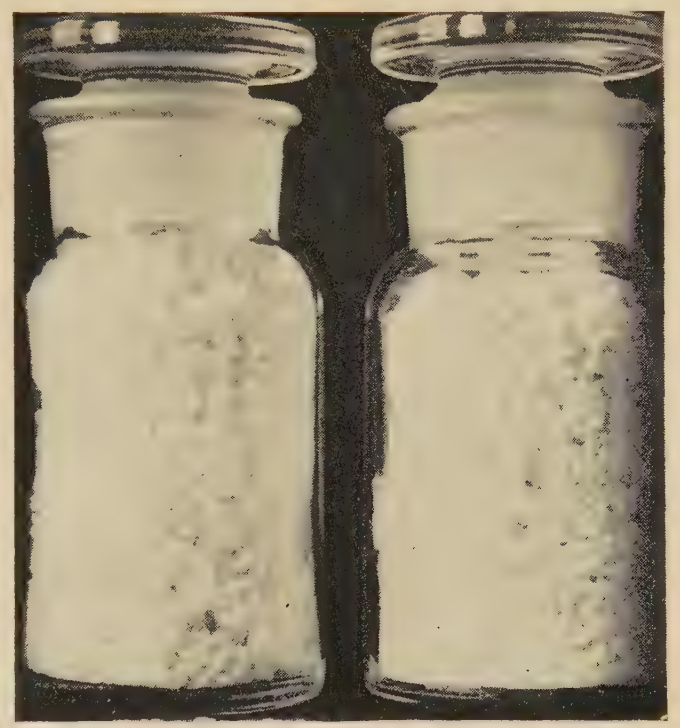

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PLATE I.

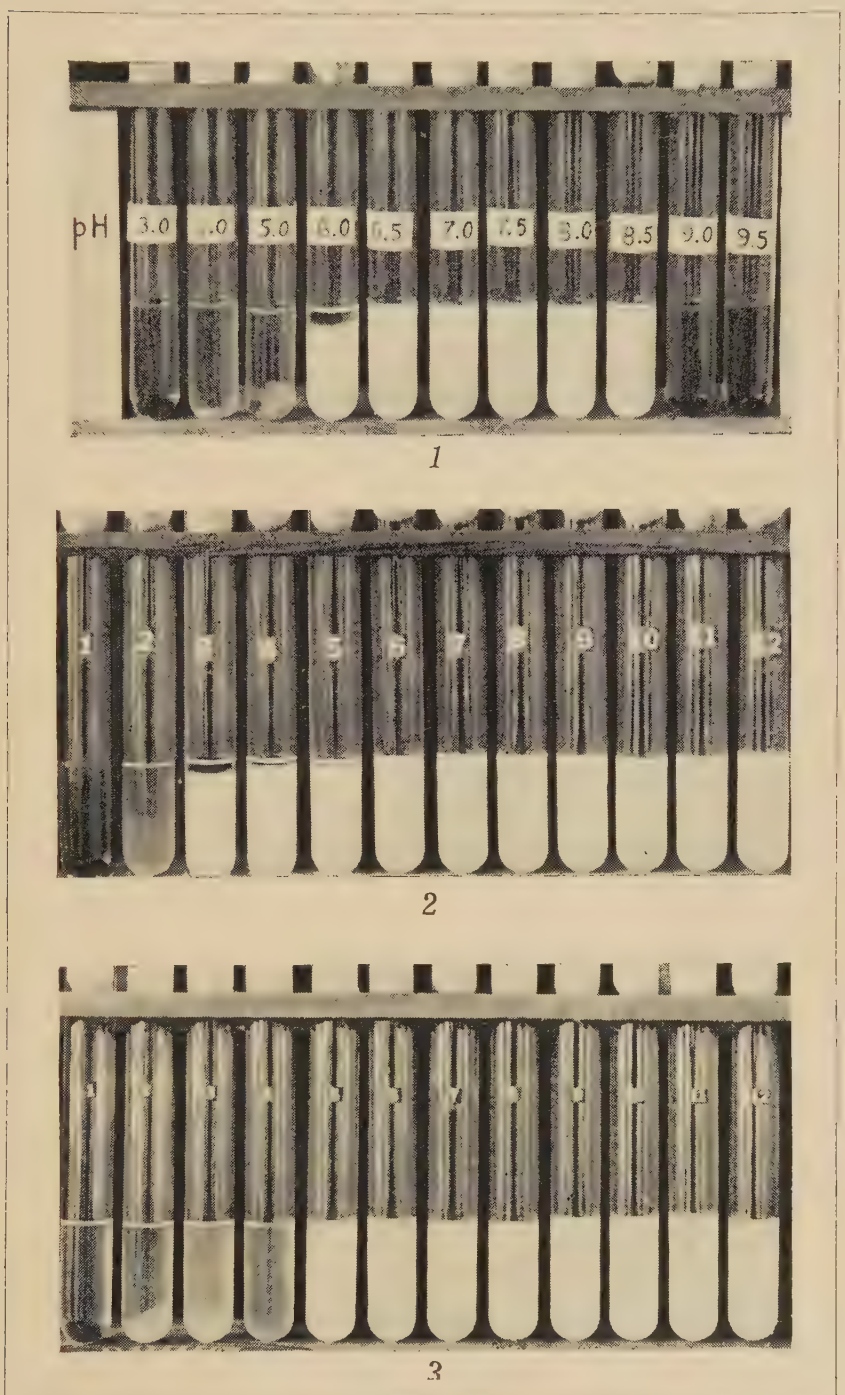

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PLATE 2.

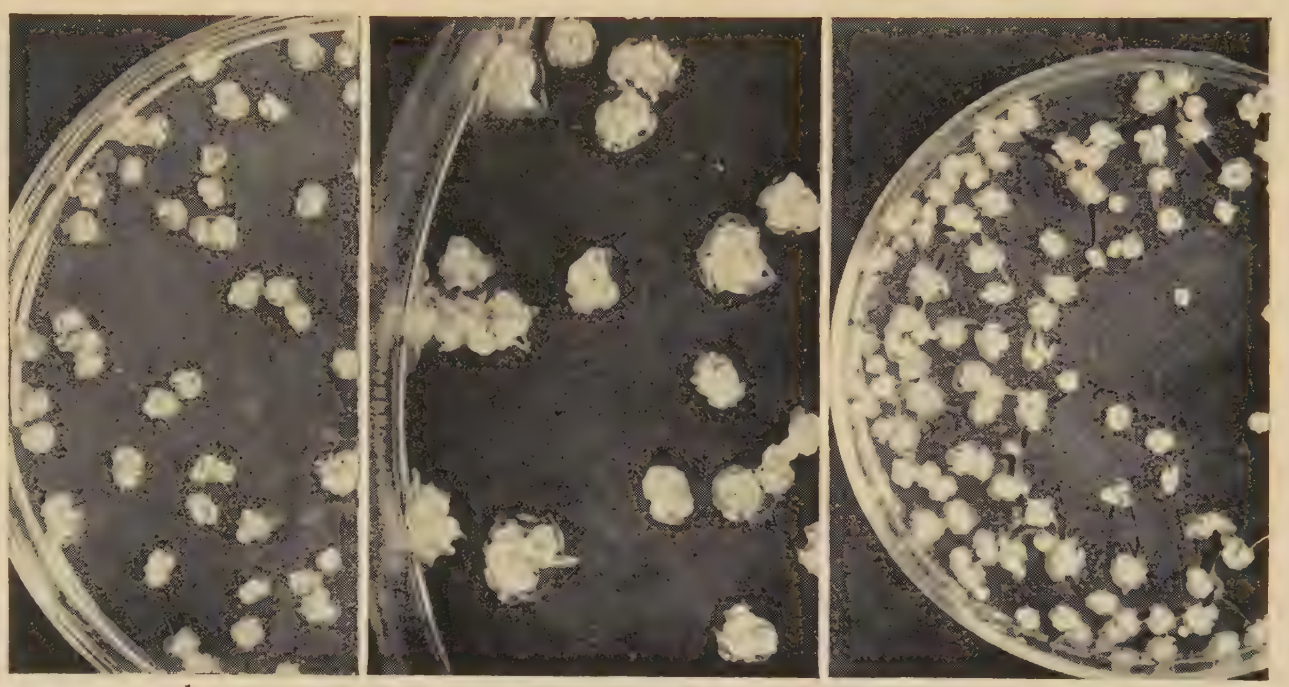

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PLATE 3.

1

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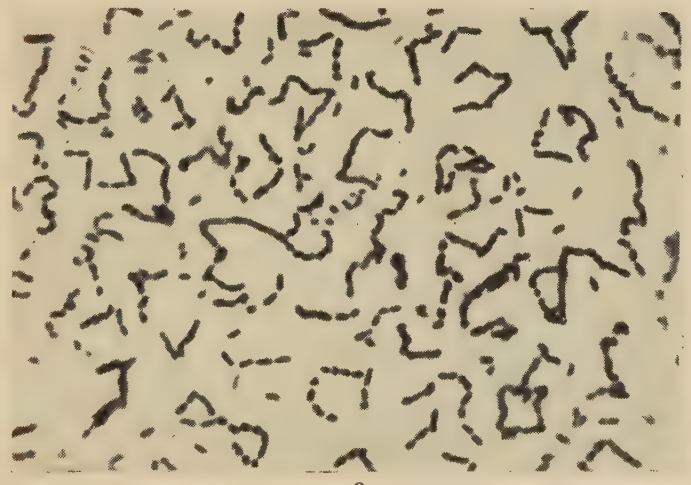

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PLATE 4.

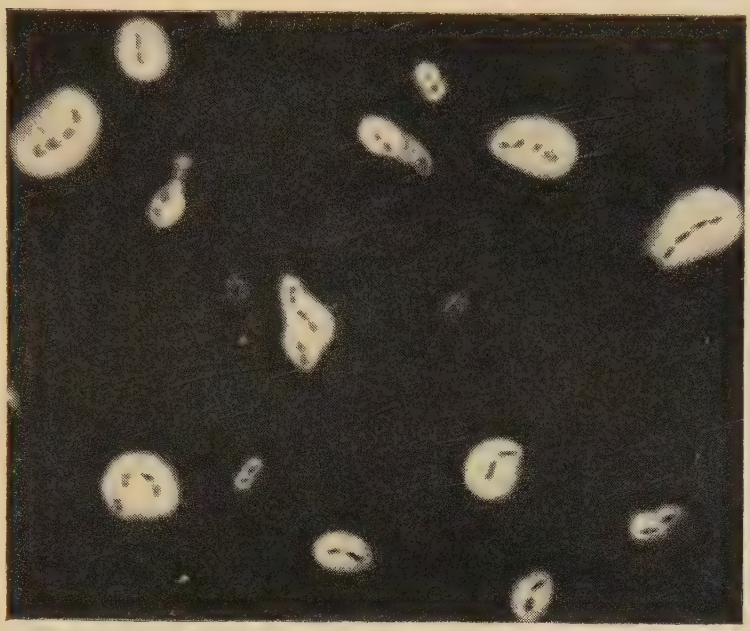

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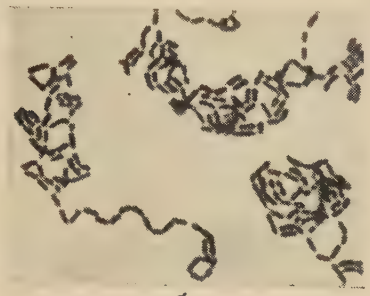
PLATE 5.



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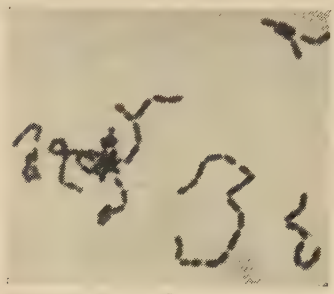

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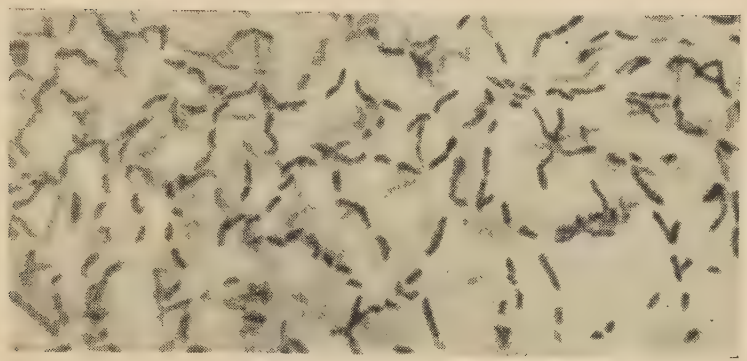

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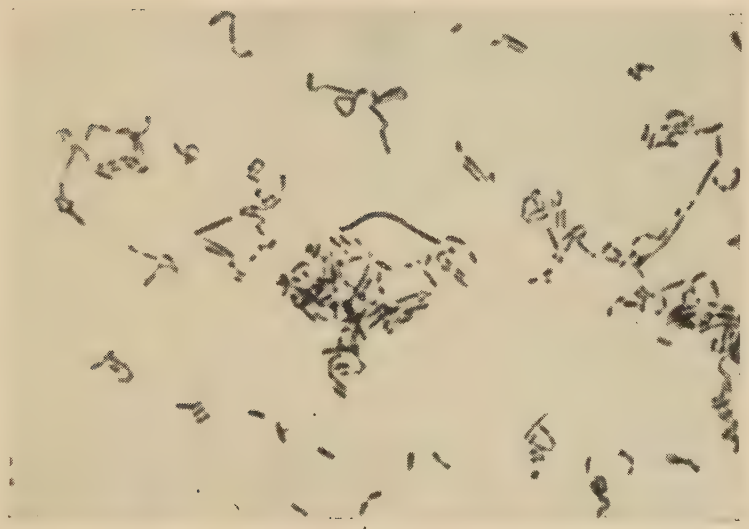

4

PLATE 6.

COMPOSITION OF PILI PULP AND PILI-PULP OIL FORM THE FRUIT OF CANARIUM OVATUM

By Lilia T. Marañon, Aurelio O. Cruz, and Angelita M. Tuason
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Several species of the genus *Canarium* grow wild in the Philippines. The fruit of the species known as *Canarium ovatum* Engl. of the Burseraceæ, which produces the largest fruit, was used in this investigation.

West and Brown [1] gives the following description of this species:

"*Canarium ovatum* is a tree reaching a height of about 20 meters and a diameter of about 40 centimeters. The leaves are alternate and compound with opposite leaflets, which are smooth, rounded at the base, pointed at the tip, and from 10 to 20 centimeters in length.

The fruits are 6 to 7 centimeters in length, and consist of hard, thick-shelled, triangular nuts surrounded by a small amount of pulp. This pulp which is edible when cooked, also contains an oil which is occasionally extracted locally and used for lighting and cooking.

This species, like *Canarium luzonicum*, yields a resinous product which is locally known by the Spanish term "brea blanca" (white pitch).

Canarium ovatum is very abundant in southern Luzon."

As shown by our analysis, pili pulp is similar in composition to avocado, and is very nutritious on account of the oil, carbohydrates and protein which it contains. The oil has similar characteristics as those of the oil analyzed by Jamieson.[2] However, its chemical composition has not yet been investigated. The results of our work show that it consists principally of glycerides of oleic, linoleic and saturated acids and resembles in composition pili nut and American cottonseed oils.

According to Juliano and Guerrero [3] traces of cyanophoric substance have been found in the pulp of the fruit. However, the substance has been found positive in the leaves, bark of stems and roots. For edible use, the black outer covering of

[1] West, Augustus P., and William H. Brown. Philippine resins, gums, seed oils, and essential oils. Philippines. Bur. Forestry Bull. 20 (1920) 12–15.

[2] Jamieson, G. S. Vegetable fats and oils. 2nd ed. New York, 1943. 508p.

[3] Juliano, Jose B., and Martin Guerrero. Philip. Agric. 24 (1935) 22–26.

the fleshy portion (pulp) is removed. Austria,[4] in studying the removal of the husk (pulp), recommended the soaking of the fruit in water at 40° to 50° C for practical and general purposes. No report of ill-effects on persons who have eaten as much as ten fruits is known. This is probably due to the fact that the cyano-genetic substance is removed in soaking the fruit in warm water, or in separating the black outer covering.

EXPERIMENTAL PROCEDURE

The fruit of *Canarium ovatum* weighs about 28 grams and consists of 64.5 per cent pulp and 35.5 per cent of a hard, thick-shelled, triangular nut.

The samples used in this investigation were kindly presented to the authors by Dr. Jose S. Reyes of Bulusan, Sorsogon, and Miss Guillermina V. Canchela of the Institute of Science and Technology. The pulp was separated by cutting the fruit longitudinally and removing the nut. It was then cut into small pieces and analyzed. Its composition compared with the composition of avocado is given in Table 1.

TABLE 1.—*Composition of pili pulp and avocado.*

	Pili pulp		Avocado	
	Fresh	Dried	Fresh	Dried
	Per cent	*Per cent*	*Per cent*	*Per cent*
Moisture	72.8		85.2	
Protein	2.9	8.0	1.6	10.8
Fat (ether extract)	12.4	33.6	8.6	58.1
Crude Fiber	1.3	3.4	2.1	14.2
Ash	3.4	9.2	0.7	4.7
Carbohydrates (by difference)	7.2	45.8	1.8	12.2
Total	100.0	100.0	100.0	100.0
Calories per kilo	1,567.3	5,330.6	939.2	6,345.1

[a] Bocobo, D. L., V. L. Pobre, and C. Ll. Intengan, Food Composition Tables. Philippines. Institute of Nutrition, Handbook 1 (1951) 22–23.
[b] Calculated.

As shown by the data, pili pulp has about the same food value as avocado. Its oil content is slightly lower than that of the pulp of the fruit from *Canarium oleosum* cited by Jamieson.[5]

[4] Austria, P. L. Philip. Agric. **20** (1931–1932) 659–668.
[5] Jamieson, *Op. cit.*

One of the commercial methods employed to obtain the oil from oilseeds and nuts is extraction with solvents. Various solvents were used to determine the more suitable for extraction purposes. The material used was the pulpy portion which was oven-dried and ground. The solvents used in this investigation to obtain the oil from the pulp were benzene, petroleum ether, chloroform, sulfuric ether, and carbon tetrachloride. In working with these solvents the usual Soxhlet extractors heated by electric heaters were employed. With the use of 2 to 3 grams of the dried pulp, extraction lasted for 12 hours. The extraction temperature was regulated so that the rate of extraction was practically the same for each solvent.

The average results obtained in the extraction of dried pili pulp with different solvents are recorded in Table 2.

TABLE 2.—*Extraction of the dried pili pulp with different solvents.*

Solvent	Extraction
	Per cent
Benzene	32.1
Petroleum ether	32.8
Chloroform	33.1
Ether sulfuric	33.4
Carbon tetrachloride	33.4

Table 2 shows that the percentage of extraction is approximately the same for benzene, petrolemu ether, chloroform, sulfuric ether, and carbon tetrachloride.

The pili-pulp oil was prepared by immersing the pili fruit in warm water until the pulp became soft. This was separated by hand from the seed, macerated in a little water and then squeezed through a piece of cloth. The liquid obtained was boiled until only the oil remained which was freed from solid matter and dried.

A portion of the crude oil was purified by adding a calculated amount of alkali solution to neutralize the free fatty acids. The oil was then separated and washed with warm water until free from alkali and dried. The refined oil was treated successively (warming, shaking and filtering) with 2 per cent Fuller's earth and 0.5 per cent activated carbon. This treatment removed the color and colloidal matter and produced a brilliantly clear greenish yellow oil. The constants of the refined pili-pulp oil compared with the oil analyzed by Jamieson are given in Table 3.

TABLE 3.—*Physical and chemical constants of pili-pulp oil.*

	Philippine	American [a]
Specific gravity at 30°C	0.9053	
Refractive index at N $\frac{20}{D}$	1.4722	1.4584
Saponification value	194.1	183.5
Free fatty acids (as oleic, per cent)	0.1	
Iodine number (Hanus)	72.2	78.2
Thiocyanogen value	61.0	
Unsaponifiable matter (per cent)	0.5	4.9

[a] Jamieson, G. S. Vegetable fats and oils (2nd ed.; New York, 1943) 160.

As shown by the data the pili-pulp oil has similar characteristics as those of the oil mentioned by Jamieson.

The saturated and unsaturated glycerides that occur in the pili-pulp oil were calculated from the iodine and thiocyanogen values of the oil in accordance with the Official and Tentative Methods of the American Oil Chemists' Society.[6] The results are recorded in Table 4, which include the compositions of pili-nut (kernel) oil and cottonseed oil.

TABLE 4.—*Composition of pili-pulp oil, pili-nut (kernel) oil and cottonseed oil.*

	Pili-pulp oil	Pili-nut (kernel) oil	Cotton-seed oil
	Per cent	*Per cent*	*Per cent*
Glycerides of:			
Unsaturated acids—			
Oleic	56.7	57.2	35.2
Linolic	13.5	4.7	41.7
Saturated acids	29.3	38.1	22.9
Unsaponifiable matter	0.5		
	100.0	100.0	99.8

The results of our test show that pili-pulp oil is similar in composition to pili-nut oil.[7] It has a higher percentage of oleic glyceride but only one-third of linolic glyceride when compared with cottonseed oil analyzed by Jamieson and Baughman.[8] All these oils consist principally of glycerides of oleic, linolic and saturated acids.

[6] American Oil Chemists' Society. Official and Tentative Methods of the American Oil Chemists' Society. 2nd ed. Chicago: The Society, 1946.

[7] West, A. P., and S. Balce. Composition of pili-nut oil. Philip. Jour. Sci. 23 (1923) 269.

[8] Jamieson, G. S., and W. F. Baughman. American cottonseed oil analyzed. Amer. Chem. Soc. Journ. 42 (1920) 1197.

SUMMARY

The pulp from pili fruit is similar in composition to avocado, and is very nutritious on account of the oil, carbohydrates and protein which it contains.

The extraction of the dried pulp with different solvents has been undertaken. The results show that the percentage of extraction is approximately the same for benzene, chloroform, petroleum ether, sulfuric ether and carbon tetrachloride.

The composition of pili-pulp oil has been determined and the results (Table 4) indicate that pili-pulp oil has a composition very similar to that of pili-nut oil and American cottonseed oil.

The percentage of oleic glyceride is higher in the pili-pulp oil than in the cottonseed oil. Pili-pulp oil has only one-third the linolic glyceride when compared with cottonseed oil, while the percentage of saturated glycerides is about the same. All these oils consist principally of glycerides of oleic, linolic and saturated acids.

Pili-pulp oil is suitable commercially for the manufacture of edible products and soaps and for other purposes for which cottonseed oil may be employed.

THE MINERAL CONTENTS OF BILIS, A MARINE FISH OF MALAYA

By Kokasat Svasti
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Among the many kinds of fish regularly displayed in the markets of Malaya, a popular variety with all races is a small marine fish of the genus *Stolephorus,* commonly called "long-jawed anchovy" in English, "bilis" in Malay and "dilis" in Tagalog (Leverton and Payawal, 1951). Many species, such as *S. Commersonii, S. heterolobus,* and *S. pseudoheterolobus,* are caught in fixed stakes built near the shores of Malaya.

The smallest species of the genus, *S. pseudoheterolobus,* is the most abundant in Singapore waters (Tham, 1951); it seldom exceeds 8 to 9 cm in length (Delsman and Hardenberg, 1934) and is always available in the markets in either the fresh, cooked or dry form, the fresh form being the least plentiful, since it begins to decompose within a few hours of catching. The greater part of the catch is cooked immediately by immersion for a few minutes in boiling sea water; the unsold surplus of the cooked form is dried in the sun, after which it is preserved almost indefinitely and sold in inland parts of Malaya.

All three forms of this staple food are popular, since its low price (about $0.10 per 100 gm) places it within reach of the lower income groups and its size permits even the poorest to buy a small quantity.

It is therefore of interest to study the chemical composition of this fish (*S. pseudoheterolobus*) in relation to the elements essential to human nutrition. The present paper describes a comprehensive chemical analysis of *bilis* obtained from various sources and prepared in several different ways.

EXPERIMENTAL

Moisture, ash, calcium, phosphorus, iron, fat and protein were estimated in the dry, cooked and fresh forms of the fish. Since the fish are eaten whole, the necessary information is obtained by analysing whole fish without previous dissection.

Program of work.—Samples of dry, cooked and fresh *bilis* were purchased at each of six markets in Singapore. The fish were sorted into four groups according to length: (1) 20 to 30 mm, (2) 31 to 40 mm, (3) 41 to 50 mm, and (4) 51 to 60 mm, giving 72 samples in all. It was not feasible to make a complete analysis of each sample, and the detailed analyses which were decided to be made were governed by two considerations: (i) about 90 per cent of the fish regularly displayed in the markets is of the smallest size group; (ii) this fish finds its most abundant sale in its dry form.

Each of the six samples of the smallest size fish in the dry form was therefore analyzed for moisture, ash, calcium, iron, and protein. Statistical analysis was applied to the results to establish the probable range of the essential elements per 100 gm of fish. On a composite of the six samples of the smallest size fish in the dry form, phosphorus and fat were determined.

For the cooked form, the six samples of the smallest size fish were mixed into a composite sample which was analysed for moisture, ash, calcium, phosphorus, iron, fat, and protein. For the fresh form, a similar sample of the smallest size was analysed for moisture, ash, calcium, phosphorus, iron, fat, and protein.

Samples of the cooked form of each of the other three sizes—31 to 40 mm, 41 to 50 mm and 51 to 60 mm—were mixed to form three separate composite samples and protein and calcium in each of these samples were estimated to determine the range of these important constituents.

Preparation of the samples.—The fresh and cooked fish began to decompose within a few hours in the climate of Singapore. Ordinary refrigeration of the fish was found unsuitable as even the chilled samples decomposed after a few days while "deep-freezing" was unsuitable because of the danger of decomposition during the subsequent thawing-out process.

Complete drying of the fish followed by grinding and sifting produced the best results; samples prepared in this manner could be preserved unchanged for several months in bottles placed in a desiccator over anhydrous calcium chloride. Drying over concentrated sulphuric in vacuo was first tried and found unsuitable as the absorption of moisture was not rapid enough to prevent putrefaction and the product could not be ground to a fine powder. Drying in an electric air oven set at 100

to 105° C was, however, satisfactory. The fish dried rapidly without putrefaction and the resulting product could be ground in a mortar to a fine powder which, after passing through a 60-mesh sieve, could be stored unchanged for long periods of time. We believe that no serious change in the original composition of the fish occurred in this method of drying apart from the loss of moisture which was estimated by weighing an aliquot of the sample before and after drying, and the loss of volatile nitrogeneous compounds, such as trimethylamine.

Sampling.—To obtain homogeneous and representative samples for each form of fish, random sampling was adopted. After each batch of fish purchased from the market had been sorted, sampling was made as follows: for the dry fish, 25 gm were taken at random from each of the six batches brought from the markets. About 5 gm of each sample were used for moisture determinations. The remainder of each lot was dried in the air oven, ground, sifted and preserved in separate bottles.

A composite sample was also prepared for the determinations of phosphorus and fat by mixing 5 gm lots from each of the six batches of fish; these were dried, ground and preserved in the same manner.

Composite samples of the cooked and fresh fish were treated in the same way but using about 50 gm of each batch of the fish. Moisture determinations were made on aliquots.

The analyses were made using the dried powdered samples: the results were expressed as a percentage of the original fish by using the moisture content data.

METHODS OF ANALYSIS

Moisture.—Moisture was determined by drying 5 to 15 gm of fish to constant weight in an air oven set at 100 to 105° C (24 hours for the dry or cooked fish, 30 hours for the fresh fish).

Ash.—Ash was estimated by igniting 2 to 3 gm of the fish powder in an electric muffle furnace, the temperature of the furnace being raised gradually to 650° C and maintained at this value for thirty minutes.

Calcium.—The method recommended by the Association of Official Agricultural Chemists for the analysis of calcium in cereal foods was adopted (1950). After ignition of the fish powder and dissolution of the residue in hydrochloric acid, calcium was precipitated as oxalate and the latter was dissolved

in dilute sulphuric acid and titrated with standard potassium permanganate solution.

Phosphorus.—The samples were carefully ignited with a solution of hexahydrated magnesium nitrate (Roepke, 1935) and phosphorus precipitated as the yellow phosphomolybdate complex in dilute nitric acid solution according to Charlot and Bézier (1949). After filtration and washing, the yellow complex was dissolved in a known volume of standard sodium hydroxide solution and titrated with standard sulphuric acid using phenolphthalein as indicator, according to the recommendation of Hillebrand and Lundell (1950).

Iron.—A slight modification of the absorptiometric method of Pringle (1946) was adopted. The method consists of measuring the optical density of the orange-red solution formed by ferrous iron on combining with 1:10-phenanthroline. The iron concentration in the test solution was then obtained by reference to a graph constructed from data obtained by making different dilutions of a standard solution of iron.

Instead of a solution of sulphur dioxide employed to reduce iron to the ferrous state, a 10 per cent solution of hydroxylamine hydrochloride was used. The measurement of the optical density of the colored solutions was made after standing for 24 hours according to Hummel and Willard (1938). Hilger's "Spekker" absorptiometer was employed to measure the optical density, together with Chance No. 603 Spectrum Blue-Green filters with a maximum transmission at 490 $m\mu$ and Hilger H. 697 Heat Absorbing glass filters. All-glass cells (capacity 25 ml) were used to contain the colored solutions which were measured in the apparatus against a blank of the reagents.

Fat.—Fat was determined by extracting 3 to 4 gm of the powdered fish samples with ethyl ether for 18 hours in a Soxhlet apparatus. The greater part of the ether was eliminated by gently heating over a hot-plate and the fat dried in an air oven to constant weight.

Protein.—Total nitrogen was estimated by a modified macro-Kjeldahl method and the protein content in the sample was taken as (Total Nitrogen) $\times$ 6.25 as advised by Nicholls (1951).

The method recommended by the Association of Official Agricultural Chemists was first followed (1950). This entails a brisk heating of the sample with concentrated sulphuric acid

and catalysts until the mixture turns colorless, followed by another period of heating for two hours. The digest is distilled, after dilution, with 45 per cent sodium hydroxide solution and the ammonia evolved is absorbed in a known volume of 0.1N sulphuric acid, the excess of which is titrated with 0.1N sodium hydroxide.

As catalysts, the mixture of elemental selenium, cupric sulphate and potassium sulphate suggested by Pregl (1951) was chosen instead of mercuric oxide or a mixture of mercury and cupric sulphate recommended by the A.O.A.C., both of which require treatment before the distillation of ammonia. About 200 mg of Pregl's catalyst mixture were used with each aliquot of powdered fish of approximately 300 mg.

After one hour of brisk boiling, the digest turned straw in color and when the rapid heating was continued for two more hours, the final digest was either green or blue. The nitrogen values in the green or blue digests were not in good agreement: a series of twelve aliquots of fish powder treated under the same conditions produced results varying from 11.40 to 11.92 per cent. A modification was therefore necessary.

Modification.—As described by Middleton and Stuckey (1951), the Kjeldahl digestion first takes place with carbonization of the sample, reduction of sulphuric acid and the evolution of sulphur dioxide and accompanying white fumes. Later, the carbon is oxidized by oxygen produced from the decomposition of sulphuric acid and eliminated as carbon dioxide and the digest becomes clear.

The use of sulphuric acid is therefore not only to cause the decomposition of the sample but also to oxidize carbonaceous matter. As stated by Kirk (1950) sulphuric acid is normally a weak oxidizing agent and to use it to oxidize organic materials, strong heating is essential. Mathieu (1946) and Lake, et al. (1951) insist that a temperature of at least 330° C must be reached.

High temperature alone is not wholly effective as the reaction is too slow. The reaction may be considerably accelerated if certain metals or compounds are added as catalysts—mercury, zinc, vanadium, selenium, molybdenum, copper and their compounds are commonly used.

While the oxidizing reaction is proceeding, the transformation of nitrogen, probably first to ammonia and then to ammonium sulphate, is also taking place. If the reaction is

carried out too violently, there is danger that nitrogen will become oxidized and a loss will occur (Kirk, 1947).

Furthermore, Kirk (1947, 1950) stated that "analysts have too often assumed that clearing of a Kjeldahl digest signifies virtual completion of the digestion, whereas a digest may clear long before the decomposition is complete." Many workers advise further gentle heating after the sample has cleared so that quantitative transformation of nitrogen is achieved. Among those who recommend this gentle heating, the following may be mentioned: Chibnall, et al. (1943), Jonnard (1945), Dalrymple and King (1945), Bradstreet (1945) and Middleton and Stuckey (1951). This period of gentle heating or "after-boil" covers 2 to 3 hours to 16 hours or even overnight.

On comparing the method employed with the ideal Kjeldahl digestion outlined above, the inconsistency of results may have been caused by: (*i*) the use of unsuitable catalysts); (*ii*) the overheating of the digest; and (*iii*) the incomplete transformation of organic nitrogen into ammonium sulphate.

As regards the catalysts, selenium has been reported by Kirk (1950) as a source of conflicting results, but Mathieu (1946) and Lake, et al. (1951) recommended it especially when a slower reaction is preferable, and Dalrymple and King (1945) considered it to be the best catalyst. As far as this work is concerned, it was not possible to examine the matter further but it may be stated that selenium was a useful indicator during the distillation stage, as the digests containing it turned muddy in appearance upon adding sodium hydroxide but gradually cleared again as the ammonia distilled off. The quantity of 7 mg of selenium used with each aliquot of powdered fish throughout this work was so far below the maximum limit of 250 mg set by Bradstreet (1945) that it appeared a change of catalysts was unnecessary.

The quantity of potassium sulphate employed was also much lower than the amount which many authors recommended (Jacobs, 1945; Pregl, 1951). Overheating of the digests was therefore unlikely.

However, the incomplete transformation of organic nitrogen into ammonium sulphate appeared to be the most likely cause of inconsistent results. It seemed, therefore, that the practice should be introduced of gently heating the digests for sometime after the initial period of brisk boiling. The optimum duration of this "after-boil" treatment should be determined and

adopted as standard for the routine analysis of the fish. It was important that the "after-boil" be applied at the right stage; if applied too early, the brisk boiling would not have time adequately to decompose the sample and low values would result; if applied too late, a loss of nitrogen would occur through prolonged brisk boiling.

Experiment to determine the beginning of the gentle "after-boil" heating.—It was observed that the straw, green and blue digests which were found to produce inconsistent results could be obtained by boiling the samples briskly for one, two and three hours respectively. If the same period of gentle "after-boil" heating was applied to each type of digests, their effects would be established and the color associated with the highest values of nitrogen could then be adopted as the indication for the beginning of the "after-boil" heating.

For this purpose, twenty-seven aliquots were weighed from the same homogeneous sample of powdered fish. The first nine aliquots were boiled briskly with concentrated sulphuric acid and the catalysts for one hour to produce the straw digests; the second lot of nine aliquots for two hours to obtain the green digests and the remaining nine aliquots for three hours for the blue digests. The same period of gentle "after-boil" heating of six hours was then applied to all the digests with a small flame which just touched the bottom of the flasks. The period of six hours was chosen according to the suggestion of Dalrymple and King (1945). Later, experiments were to be made to select the most suitable period of "after-boil" by varying the time to cover from 4 to 10 hours.

The results appear in Table 1 where the nitrogen values show a marked increase with the period of brisk boiling as the color changed from straw to blue. It appeared that the full conversion of organic nitrogen to ammonium sulphate required brisk boiling of the samples at least till the color of the digests became blue.

To ascertain that the maximum nitrogen figures had been realized three more aliquots were weighed from the same sample of powdered fish and boiled briskly for four hours. Gentle "after-boil" heating of six hours was applied as before and the results, which also appear in Table 1, show a distinct loss of nitrogen and indicate that brisk boiling should be stopped after three hours.

TABLE 1.—*Effect of time of brisk boiling on color of digests and observed nitrogen content of aliquots.*

("After-boil" period = 6 hours)

Time of brisk boiling and color of digest	Observed nitrogen (gm/100 gm of dried powdered fish)			
	First set	Second set	Third set	General mean
1 Hour	11.56	11.52	11.50	----------
Straw	11.61	11.45	11.59	----------
Mean	11.54	11.40	11.69	----------
	11.57	11.46	11.59	11.54
2 Hours	11.86	11.52	11.61	----------
Green	11.81	11.70	11.69	----------
Mean	11.92	11.72	11.79	----------
	11.86	11.65	11.67	11.73
3 Hours	12.22	12.17	12.07	----------
Blue	12.11	12.21	12.02	----------
Mean	12.25	12.15	12.14	----------
	12.19	12.18	12.08	12.15
4 Hours	11.92	----------	----------	----------
Blue	11.89	----------	----------	----------
	11.92	----------	----------	11.91

Experiment to select the optimum period of gentle "after-boil."—Seventeen aliquots of the same sample of powdered fish were heated briskly for three hours until the digests became blue. Gentle heat was then applied to sets of three aliquots for the time varying from 4 to 10 hours. The nitrogen values are set out in Table 2 below:

TABLE 2.—*Effect of the variation of the "after-boil" period.*

(Gm nitrogen per 100 gm of powdered fish)

Period of "after-boil"	Aliquot 1	Aliquot 2	Aliquot 3	Mean
Hours				
4	11.52	11.58	11.61	11.56
5	11.71	11.70	11.72	11.71
6	12.12	12.23	12.10	12.15
7	12.00	12.13	12.03	12.05
8	11.92	11.95	11.93	11.93
10	11.64	11.76	----------	11.70

The figures show that any period of "after-boil" under 6 hours will not ensure the complete transformation of nitrogen into ammonium sulphate and results will be low, while any period greater than 6 hours causes a progressive loss of nitrogen although the loss caused by an "after-boil" period of 7 hours still falls within a fairly acceptable limit.

It may be formulated that for the best conditions in the determination of Kjeldahl nitrogen in the fish, the samples should be digested with concentrated sulphuric acid and the catalysts mixture for nine hours, divided into the intervals of three hours for the brisk boiling and six hours for the gentle "after-boil" heating.

Procedure.—Instead of the pear-shaped flasks, round-bottom flasks of one liter capacity and with neck of at least 17 cm long and 3.5 cm in diameter were employed for the digestion of the fish samples. The advantage in using these flasks was that the distillation of ammonia could be carried out in them so that transferring of digests, a common source of error, was eliminated.

The distillation unit consisted of a splash-head connected to a descending condenser with the inner tube of about 1 cm in diameter. The lower end of the condenser was slightly drawn out and cut to make a small tube and this end was immersed in the standard acid solution contained in a conical beaker 15 cm high. The digests were distilled for 75 minutes of which 30 minutes were for the slow distillation at the beginning, 30 minutes for the rapid distillation and 10 to 15 minutes for washing down the condenser. The acid solution was titrated with standard sodium hydroxide solution until the methyl red indicator turned yellow.

DISCUSSION

The analytical results of the dry, cooked and fresh fish, expressed as percentages of the weight of the fish as purchased from the markets, are set in Table 3. Table 4 presents the statistical analysis of the results for the dry fish in Table 3, showing the probable range of values to be expected from a repetition of similar analyses. The confidence intervals have been calculated from values of "t" at the 5 per cent level for the appropriate number of degrees of freedom (Youden, 1951). The Table shows that 100 gm of dry fish which cost about 30 Malayan cents, at least 29.1 gm of moisture and 16.6 gm of ash are present. In the same sample, not less than 45.3 gm or protein, 0.98 gm of calcium, and 8.5 gm of iron may be expected.

To examine the nutritional value of the fish, the mean contents of protein, calcium, iron, phosphorus and fat of the dry, cooked and fresh *bilis* are compared in Table 5 with the daily

allowances recommended by the British Medical Association (1950), the U. S. National Research Council (1948), and the dietary allowances under tropical conditions of Nicholls (1951), Nutrition Adviser to the Commissioner General, South East Asia. The British and American allowances are based upon the daily requirements of an active man weighing 70 kg, whereas those of Nicholls are based on the needs of an active man weighing 55 kg. The figures for the fish are gm per 100 gm of fish as purchased from the markets.

The average protein figure of 45.8 gm of the dry *bilis* compares favorably with the Dietary Allowances of Nicholls. In this case, 100 gm of fish contribute approximately 70 per cent of the daily requirement of protein. Compared with the British and American allowances, the dry fish contributes 52 per cent and 65 per cent respectively of the daily requirement. According to Rosedale (1935) who analysed several kinds of dry fish normally available in Malayan markets, the dry *bilis* contains more protein (45.4 per cent) than larger dry fish such as the pomfret (34.8 per cent) or the horse mackerel (36.4 per cent).

Table 5 also provides figures for the protein contents of cooked and fresh *bilis*. The quantities compare well with those of local fresh meats and sea foods considered as good sources of protein, such as beef (19.9 per cent), prawn (23.7 per cent), crab (13.2 per cent), and oyster (9.2 per cent). When it is remembered that the *bilis* is eaten whole with no waste of bones, shells or fins, and that 100 gm of either form costs only a few cents, it will be seen that this little fish provides an excellent and cheap source of protein.

The range of protein contents are set in Table 6. The figures show that the largest size fish examined contains a little more protein than the smallest size.

As was to be expected from the fact that *bilis* is eaten whole, all three forms of the fish are good sources of calcium. According to Table 5, 100 gm of dry *bilis* provide all the daily requirement stipulated by the American allowances, more than the requirement recommended by the British Medical Association and twice the dietary allowances under tropical conditions. The cooked fish contributes about half of the allowances set by British and American authorities and provides all calcium stipulated by Nicholls.

TABLE 3.—*Composition of dry, cooked and fresh fish.*

(Gm/100 gm of fish as purchased from the markets)

Constituents	Dry fish						Cooked fish	Fresh fish
	Market							
	1	2	3	4	5	6		
Moisture	31.17	28.96	36.91	31.55	25.86	29.80	68.13	84.06
Ash	15.90	17.58	17.88	16.48	16.90	18.52	8.23	1.50
Calcium	0.93	0.87	1.17	1.13	1.07	1.05	0.47	0.26
Iron	0.0166	0.0072	0.0109	0.0057	0.0165	0.0092	0.0035	0.0011
Protein	46.10	45.12	45.34	45.60	47.22	45.61	22.01	13.23
Phosphorus	--------	--------	--------	1.15	--------	--------	0.43	0.29
Fat	--------	--------	--------	4.02	--------	--------	2.00	0.98

Phosphorus and fat in the dry fish were determined in the composite sample of the six markets.

TABLE 4.—*Statistical analysis of experimental results for dry fish in Table 3.*

Constituents	Mean	Standard error of mean	Degrees of freedom	Range with 95 per cent confidence
	Per cent	*Per cent*		*Per cent*
Moisture	30.71	0.77	17	29.1 to 32.3
Ash	17.21	0.26	11	16.6 to 17.8
Protein	45.83	0.24	16	45.3 to 46.3
Calcium	1.03	0.026	17	0.98 to 1.08
Iron	0.0114	0.0013	11	0.0085 to 0.0143

The figure of 260 mg of calcium per 100 gm of fresh *bilis*, though far below the recommended allowances, is more than twice the content of calcium (120 mg) in whole cow's milk (Nicholls, 1951; Bridges and Mattice, 1942). It is therefore reasonable to consider all three forms of *bilis* as a better source of calcium than fresh milk which is rather expensive in this part of the world. The fact that this fish can replace milk as a source of calcium has been proved by Leverton and Payawal in the Philippines (1951) who employed the powdered form of another species of the *bilis* family (*S. Commersonii*) to feed children on a diet completely free from milk.

The range of calcium contents in Table 6 shows that the largest size fish examined contains nearly double the amount of calcium of the smallest size fish.

According to the mean content of iron presented in Table 5, it appears that the dry *bilis* is an excellent source of this im-

portant constituent. However, the results of separate analyses of six samples in Table 3 show such a wide difference in the iron content that it is difficult to accept the mean as representing the natural content of iron in the fish. As stated by Bridges and Mattice (1942), the natural content of iron in food stuffs is often augmented by contact with containers or articles made of the metal, and it seems possible that the wide variation in the iron content in dry *bilis* may be due to this reason. The dry form is made by drying the unsold surplus of the cooked form which, in turn, is prepared by boiling the fresh fish in sea water contained in an iron cauldron. Moreover, the drying process invites contamination from iron as large quantities of *bilis* are dried in the sun, placed on low concrete slabs and turned at intervals with an iron spade.

TABLE 5.—*Nutritional value of dry, cooked and fresh bilis compared with recommended daily allowances.*

Constituents	gm/100 gm of fish as purchased from markets			Recommended daily allowances		
	Dry *bilis*	Cooked *bilis*	Fresh *bilis*	British Medical Association (1950)	U.S. Nat. Research Council (1948)	Nicholl's Tropical Allowances (1951)
				gm	*gm*	*gm*
Protein	45.83	22.01	13.23	87.0	70.0	65.0
Calcium	1.03	0.47	0.26	0.8	1.0	0.5
Iron, mg	11.40	3.50	1.10	12.0	12.0	8.0
Phosphorus	1.15	0.43	0.29	1.3	1.2	Variable
Fat	4.02	2.0	0.98	Variable	Variable	50.0

TABLE 6.—*Range of protein and calcium contents of cooked fish.*

(Gm/100 gm of fish)

Size in mm	Protein	Calcium
20 to 30	22.01	0.47
31 to 40	22.32	0.55
41 to 50	24.01	0.75
51 to 60	24.17	0.82

The increase in iron content during cooking and drying is demonstrated by a comparison of the figures for the moisture-free fish in its three forms. We find that the fresh fish, after oven-drying in the laboratory, contains 6.9 mg of iron per 100 gm while the corresponding figures for the cooked and dry fish are 10.9 and 16.5 mg respectively. These increases are too

great to be explained by loss of other constituents during processing and must indicate contamination.

The natural iron content of dry *bilis* ought to be less than the value reported in Table 5 and the quantity of 6 mg per 100 gm of fish, given by Nicholls for dry whitebait (1951), is more likely to represent the true food value of the *bilis*. Even this lower figure places it among excellent sources of iron foods, such as mussels (6 mg) and oysters (7 mg) reported by Nicholls.

The iron found in the fresh fish is probably all the natural occurrence and places this form in the same category as tinned sardines (1 mg), tinned salmon (1 mg), and sprats (1 mg). The cooked form, which is intermediate between the fresh and dry forms, is also a reasonable nutritional source of iron.

Of the other nutritional elements examined, Table 5 shows that the *bilis* provides a moderately good supply of phosphorus but is insignificant as a source of fat.

The importance of *bilis* as an item of the diet of Malayan peoples may be summarized by saying that it is a cheap and readily available source of protein, calcium, iron and phosphorus.

SUMMARY

The mineral content of *Stolephorus pseudoheterolobus*, a small marine fish found in Malayan waters, has been studied. The fish is called "long-jawed anchovy" in English, "bilis" in Malay, and "dilis" in Tagalog. The species studied grows to a maximum length of 8 to 9 cm and the smaller ones are regularly eaten whole.

All the three forms of fish available in the markets of Singapore, i.e. dry, cooked and fresh, have been analysed for moisture, ash, calcium, iron, phosphorus, protein, and fat.

In the course of the estimation of protein, a detailed examination was made of the influence of experimental conditions upon the Kjeldahl nitrogen figure. The optimum conditions for a quantitative yield of nitrogen and good reproducibility of results were determined.

A comparison of the analytical results with human nutritional requirements as valuated by several authorities shows that *Stolephorus pseudoheterolobus* is a good source of protein, calcium, iron and phosphorus, but of little value for its fat content.

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THE GUITARFISHES, FAMILY RHINOBATIDÆ, OF THE PHILIPPINES AND ADJACENT SEAS

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THREE PLATES AND ONE TEXT FIGURE

The guitarfishes are large to very large rays found along all Philippine coasts. Their size and appearance make them of interest to the general public, and their economic value makes them worthy of consideration, but their mode of living and habits prevent them from being well known to most people. They form the natural family Rhinobatidæ, composed of large sharklike rays found in all seas except those of arctic and subarctic waters.

The Rhinobatidæ are long, large-headed fishes with a depressed and somewhat sharklike body. Like all the other rays, the body is wider and flatter than in any of the sharks, and is more or less disklike anteriorly. The pectoral fins are widened and enlarged to form the sides of the disk, which passes gradually into the long, strong, depressed tail. The pectorals never extend forward beyond the sides of the head or to the tip of the snout, which is more or less extended. There is a conspicuous fold of skin along each side of the tail. The numerous small teeth form a pavement, adapted to crushing the Crustacea and Mollusca which form their principal food. The large eye has a projecting shield. Spiracle large, close to eye. The large oblique nostrils are of characteristic shape in each genus and there are no grooves connecting the nostrils and mouth. To high pointed dorsal fins. Caudal fin well developed, of small to medium size, sometimes lunate. The scaled body is generally smooth, but warty or bony tubercles or spines may be present on the back and shoulders and about the eyes and spiracles. Of the nine genera recognized by Norman in his monograph, three are found in the Philippines.

Guitarfishes live on the sandy or muddy bottom of bays, harbors, estuaries, and quiet seas without strong currents. They are found at all depths, from a couple of meters down to 150 meters or more. They feed upon shrimps, crabs, and

other Crustacea, various small mollusks, and on small fishes. They are often caught with hook and line and some species are desperate fighters, giving the angler a hard struggle. Large ones are prone to break the tackle and escape. In some regions they are eagerly sought by anglers who wish to catch a fish that will test their skill and strength. They are fished for on the bottom, from wharfs, or pantalans, docks or breakwaters, and from boats. They are also taken by seining and are often caught in fish corrals, or *baklad*, and in fish traps, or *bobos*. In recent years many have been taken by both beam and otter trawlers in bays, gulfs, and the open sea. The development of trawling in the Philippines has not only revealed the presence of guitarfishes where they had not been taken before, and of species hitherto unknown in our waters, but has shown them to be more abundant than formerly supposed.

In color they are all more or less gray, varying to dull brown or bluish gray, the under surface white or whitish. Some kinds are more variegated, having small spots or rings scattered over the upper surface, each enclosing a black spot; others have ocelli or eyelike spots or white with black spots and bars. With age the spots tend to disappear.

Key to the genera of Philippine Rhinobatidæ

A. Origin of the first dorsal over the ventrals, which are considerably behind the pectorals; pectorals do not extend forward beyond the mouth; a well developed subcaudal fin.
  B. Snout narrow, elongate; mouth not waved; nostril length about equal to distance between the nostrils; spiracles with two folds on their hind margins ..... *Rhynchobatus.*
  BB. Snout very broad and rounded; mouth arched forward and deeply waved; nostril length less than distance between the nostrils; spiracles without folds ..... *Rhina.*
AA. Origin of the first dorsal well behind the ventrals, which are close to the pectorals; subcaudal indistinct and little developed; pectorals extend forward beyond the mouth but not upon the snout; nostrils oblique; spiracles with one or two folds ..... *Rhinobatos.*

Genus RHYCHOBATUS Müller and Henle

Rhynchobatus MÜLLER and HENLE, Ber. Akad. Wiss. Sitz. (1837) 16, type *Rhinobatus lævis* Bl. and Schn.; GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 268; FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 300.

Rhynchobatis MÜLLER and HENLE, Syst. Beschr. Plagiostomen (1841) 111, type *Rhinobatus lævis* Bl. and Schn.

The members of this genus are large to very large rays of the western Pacific and Indian oceans, and the west coast of Africa. Of the three species recognized, only one is widely distributed and occurs in Philippine waters.

Shoulder=spot guitarfish
Arado—pating sodsod

RHYNCHOBATUS DJIDDENSIS (Forskål).

The commonest guitarfish in the Philippines and adjacent seas is the shovel-nose, or white-spotted, guitarfish (*Rhynchobatus djiddensis*). It is the *pating sodsod* of Manila Bay and much of Luzon. The Ilocanos know it as *immaradu,* and it is *arado* to most Bikolanos, while others call it *robarob.* The usual Visayan name is *bariwan,* but it is *sorsor* in the Banton dialect. The Kuyanos call it *kamansi-an* and *paitan.*

The *pating sodsod* reaches a length of three meters and a weight of more than 225 kilos, but the largest Philippine specimens I have seen were about two and a half meters long and weighed between 75 and a hundred kilos. This fish is often seen in fish markets throughout the Philippines, from Aparri to Sitankai, though it is rare to see one for sale more than a meter in length. The flesh is of excellent quality and is eaten both fresh and dried. The oil of the liver is much esteemed in some regions. The dorsal fins are dried for the Chinese trade and make a fine quality of shark's fin soup.

The elongate narrow snout is contained four to five times in the total length, excluding the caudal fin, and is proportionally shorter in adults than in the young. The head is 2.25 to 2.8 times in the length. The snout is about 1.6 times in the head, the eye 9.5 to 10.5 times and about 7 times in the snout. The teeth are in 40 to 42 rows in each jaw in small specimens; Garman gives 63 rows in the upper jaw of a large specimen. The nostrils are large, oblique, their length equal to the distance between their lower or inner ends, and three-fourths of the width of the mouth, each nostril with a short lobe on its upper or anterior valve. The spiracles nearly equal the eyes, and are close behind them, each with two folds on the hind margin. The origin of the first dorsal is over that of the ventrals and somewhat distant from the pectorals; the second dorsal is nearer the caudal than the origin of the first dorsal. As in all the guitarfishes the body and depressed tail merge imperceptibly behind the pectorals, with a small dermal

keel on each side of the tail, its origin above the end of the ventrals.

There is a row of small spines or tubercles over the backbone, two short rows on each shoulder, the inner row larger, a row above each eye from the front margin around to the spiracle above the inner orbital margin.

The *arado* is a grayfish, its body sprinkled with small white spots; over each shoulder girdle there is a black or dark brown spot equal to or smaller than the eye, and surrounded by a ring of white spots. The under side is more or less whitish to gray. Some large specimens are said to become grayish brown, but all I have seen had the characteristic markings.

The shovelnose is a famous angling fish, its size, strength, and fighting spirit making it prized by sport fishermen of the Indian Ocean and east coast of Africa. Few anglers are able to land a large specimen. To Philippine anglers seeking new thrills I suggest going after this fish, using meat or fish for bait. Favorable localities would be the Sitankai area, Malampaya Sound, Ragay Gulf, the entrance to Manila Bay, and the China Sea from Fortune Island to Subic Bay.

From the Philippines and East Indies it is found north to Japan, east and south to New Guinea and the Louisade Archipelago, and tropical Australia; it is on the coast of Indo-China and from Malaya extends to Ceylon, India, the Red Sea, and the whole east coast of Africa as far south as Natal.

Genus RHINA Block and Schneider

Rhina Bloch and Schneider, Syst. Ichth. (1801) 352, type *Rhina ancylostomus* Bl. and Schn.; Fowler, U. S. Nat. Mus. Bull. 100 13 (1941) 298.

Rhamphobatis Gill, Lyceum Nat. Hist. New York Ann. 7 (1861) 407, type *Rhina ancylostoma* (Bl. and Schn.); Garman, Mus. Comp. Zool. Mem. 36 (1913) 267.

The chief features of this genus are the short, broad, bluntly rounded snout and the deeply waved mouth, which is arched forward. Spiracles large, without folds, the nostrils nearly transverse. The pectorals are united with the head and trunk to form a subtriangular disk which is about a third of the total length and merges into the tail portion. Ventrals remote from the pectorals.

Only one species is known, confined to the warmer parts of the Indo-Pacific.

RHINA ANCYLOSTOMA Block and Schneider. **The round-snout angelfish.**

Rhina ancylostomus BLOCH and SCHNEIDER, Syst. Ichth. (1801) 352, *plate 72*, Coromandel; Indian Sea; GRAY, Illus. Indian Zool. 2 (1832–34), *plate 102, fig. 2*, India; MÜLLER and HENLE, Plagiostomen (1841) 110; GRAY, List Fishes Brit. Mus. (1851) 92, Madras; Penang; China; BLEEKER, Verh. Bat. Gen. 24 (1852) 56, Plagiostomen; Batavia, Java; WHITLEY, Fishes Australia, Part. 1. Sharks (1940) 179, *figs. 205–206*, Queensland and Broome, Western Australia.

Rhina ancylostoma VAN HASSELT, Algem. Konst. en Letterbode (1823) Java; JORDAN and FOWLER, U. S. Nat. Mus. Proc. 26 (1903) 643, Kinkwazan, off Matsushima Bay, Japan; NORMAN, Zool. Soc. London Proc. (1926) 943, *fig. 1 A*, east Africa; Red Sea; Indian Ocean; Indian Archipelago; Australia; Japan; FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 299, after Day; the Philippines and 12 other localities; HERRE, Check List Philip. Fishes (1953) 35, China Sea, southwest of entrance to Manila Bay.

Rhamphobatis ancylostomus DUMERIL, Hist. Nat. Elasmobr. 1 (1865) 482; ANNANDALE, Indian Mus. Mem. 2 (1909) 10, *pl. 5, fig. 5*, off Orissa; off mouth of River Hugli; GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 267, Africa; China; East Indies; CHEVEY, Inst. Oceanogr. Indo-Chine, 19e note (1932) 7, Cochin-China.

Rhynchobatus ancylostomus GÜNTHER, Fishes Brit. Mus. Cat. 8 (1870) 440, Madras; China; Seychelles; Penang; DAY, Fishes India (1878) 730, *pl. 193, fig. 3*, Madras; KENT, Great Barrier Reef (1893) 305; Queensland; TIRANT, Service Oceanogr. Peches Indo-Chine, 6e note (1929) 72, Cochin-China.

This is the easiest of our guitarfishes to recognize, for no other has its unique short broadly rounded or semicircular snout. Teeth in 77 rows above, 75 in the lower jaw. Spiracle as large as the eye, without folds. Nostrils half as long as width of mouth, and 1.5 times in the distance between the nostrils. The disk is almost a third of the total length, broader than its length. The first dorsal is large, triangular, the second dorsal perceptibly smaller; ventrals small; caudal rather large, lunate, the upper lobe larger; tail depressed, with a keel on each side.

A row of large spines or tubercles extends along each rostral ridge and above the inner margin of each eye to the nape; a row of still larger ones is along the middle of the back, but does not extend to the first dorsal; two more short parallel rows are over each shoulder; smaller tubercles are on the ridge before the eye.

The color is grayish or gray, the caudal paler, the entire upper surface except the head covered with white or nearly white spots which extend upon the dorsals, pectorals, ventrals, and caudal; curving lines and bars of black are more or less prominent on the head, pectorals, and disk. The under side is whitish. Some authors state that adults are uniform brown, but the color notes given are from a specimen two and a half meters long.

The round snout feeds largely on shrimps, crabs, and other Crustacea, and is said by some to be a scavenger, but there is no direct evidence of this. The flesh is good and the dorsal fins are the very finest of all for that Chinese epicurean delight, sharkfin soup.

The only specimen positively identified by me from Philippine waters was the one referred to above. It was taken by otter trawl at a depth of 35 fathoms in the China Sea, off the south entrance to Manila Bay. For a good many years I have puzzled over the source of the white-spotted "shark" fins which are the choicest and most expensive of all the fins used for sharkfin soup. These fins, seen on the drying racks at Sitankai, Siasi, and all through the Sulu Islands and along the coast of Borneo, and in the shops of the Chinese dealers in Jolo and Zamboanga, could not be those of any known shark and I could never get hold of the fish from which they are taken. But now it is plainly evident that they are the fins of this ray, the round-snout. It is therefore commonly taken by the Bajau and Samal fishermen from Sitankai to Palawan and Mindanao. It cannot be said to be abundant in Philippine waters but is certainly not a rare fish. Because its fins fetch the highest price it is a fish of some economic value. When normal trade relations are re-established, the trade in shark fins should be placed on a sound basis, with proper regulations enforced to secure fins of the highest quality. Without doubt it could be secured in larger quantities and its dried fins add considerably to the income of Sulu Province.

Nothing definite seems to be known about whether this fish furnishes sport to anglers or not. Outside the Philippines and East Indies the round-snout angelfish is known along the Asiatic coast from China and southern Japan to Indo-China and Malaya, southeast to Australia, westward to India, Ceylon, the Red Sea, and the east coast of Africa.

Guitarfishes

Genus RHINOBATUS Linck

Rhinobatus LINCK, Magazin Neustes Phys. Naturgas. Gotha, ser. 3 6 (1790) 32, type *Raja rhinobatos* Linnæus; FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 304.

Rhinobatus BLOCH and SCHNEIDER, Syst. Ichth. (1801) 353; MÜLLER and HENLE, Plagiostomen (1841) 113; GÜNTHER, Catalog Fishes 8 (1870) 441, in part; DAY, Fishes India (1878) 731; GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 269, in part; NORMAN, Zool. Soc. London Proc. 62 (1926) 944.

Leiobatus RAFINESQUE, Carat. animal plante Sicilia (1810) 16, type *Leiobatus panduratus* Raf.

Squatinoraja NARDO, Osserv. Agg. Adriaticæ Ittiol. (1824), type *Squatinoraja colonna* = *Rhinobatus columnæ* Müller and Henle.

Leiobatis BLAINVILLE, Faune Franc., Poissons (1825) 43, type *Leiobatus panduratus* Raf.

Rhinobatis BLAINVILLE, Faune Franc., Poiss. (1825) 47.

Rhinobates SWAINSON, Nat. Hist. Fishes 2 (1839) 193.

Syrrhina MÜLLER and HENLE, Plagiostomen (1841) 113, in part.

Glaucostegus BONAPARTE, Cat. metodico pesci Europei (1845) 14, orthotype *Glaucostegus cemiculus* Bonaparte = *Rhinobatus cemiculus* Geoffroy St. Hilaire.

Key to the species of Rhinobatos in the Philippines and adjacent seas

A. (*Rhinobatos*) Forward nasal valve does not extend inward upon space between nostrils, or barely does so.

B. Length of nostril is twice or almost twice in width of mouth, and about equal to space between nostrils.

C. Snout long, narrow, its length 2.6 to 3.25 times width of mouth; rostral ridges parallel, very close together except near base. *R. granulatus.*

CC. Snout broad, triangular, its length 1 and ⅝ times width of mouth; rostral ridges more or less separated for entire length; distance between spiracles 2.2 to 2.3 times in distance from eyes to snout tip ..... *R. obtusus.*

BB. Length of nostril less than twice in width of mouth and more than distance between nostrils.

D. Snout distinctly enlarged at tip; nostril length 1.25 times in width of mouth and about 1.75 times the space between nostrils ..... *R. thouin.*

DD. Snout not enlarged at tip.

E. Nostril length nearly equal to width of mouth and 2.3 to 2.5 times greater than distance between nostrils; preoral length of snout about 3 times width of mouth..... *R. armatus.*

EE. Nostril length 1.3 to 1.6 in width of snout and in distance between nostrils; preoral length of snout 2.6 to 2.66 times in width of mouth..... *R. halavi.*

AA. (*Leiobatus*) Forward nasal valve extends inwards more or less upon the space between nostrils.

F. Anterior nasal valve extending inwards but little beyond inner edge of nostril and falling much short of reaching its opposite.

G. Rostral ridges more or less separated along whole length except at tip of snout; distance from outer extremity of nostril to lateral edge of snout slightly less than length of nostril ..... *R. formosensis.*

GG. Rostral ridges very close together for anterior two-thirds of length; distance from outer rim of nostril to lateral edge of snout more than nostril length. *R. schlegeli.*

RHINOBATOS GRANULATUS Cuvier. **Rough-backed guitarfish.**

Rhinobatus granulatus CUVIER, Régne Animal, ed. 2 2 (1829) 396; BLYTH, Asiatic Soc. Bengal Journ. 29 (1860) 36, Calcutta, India; GÜNTHER, Cat. Fishes 8 (1870) 443, in part; MARTENS, Preuss. Exped. Ost-Asien 1 (1876) 409; Bangkok, Siam, and Singapore; DAY, Fishes India (1878) 732, *pl. 192, fig. 2,* Malabar; in part; VOLZ, Revue Suisse Zool. 12 (1904) 484, Padang and Kwala, Sumatra; GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 272, India; NORMAN, Zool. Soc. London Proc. (1926) 949, *text fig. 3,* type of *Rhinobatos spinosus,* Madras; India; Shanghai; coasts of India, Ceylon, China; PHILLAY, Nat. Hist. Bombay Journ. 33 (1929) 352, Travancore, India; CHEVEY, Inst. Oceanogr. Indo-Chine, 19e note (1932) 7, Indo-China; HORA and MUKERJI, Indian Mus. Records 38 (1936) 18, 21, *pl. 2, fig. 1,* Maungmagan, Burma.

Rhinobatus (*Rhinobatus*) *granulatus* MÜLLER and HENLE, Plagiostomen (1841) 117, *pl. 38,* Tranquebar and Pondicherry, India; BLEEKER, Verh. Bat. Gen. 25 (1853) 82, Bengal; DUMERIL, Hist. Nat. Elasmobr. 1 (1865) 493, Malabar, Ganges River, and Pondicherry, India.

Rhinobatus tuberculatus (Cuv.) BLEEKER, Verh. Bat. Gen. 25 (1853) 82, Bengal.

Rhinobatus spinosus GÜNTHER, Cat. Fishes 8 (1870) 518, the type locality, "Mexico," is an error.

Rhinobatus thouini DAY, Fishes India (1878) 732, *pl. 190, fig. 4,* Andaman Islands; not of Shaw.

Rhinobatis granulatus ANNANDALE, Indian Museum Mem. 2 (1909) 14, off Orissa, Bay of Bengal.

Rhinobatus acutus GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 273, *pl. 17 b, figs. 1–2,* Ceylon.

Rhinobatos granulatus FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 316, Cebu; west coast of Palawan; Sandakan; Port Moresby, New Guinea.

The long pointed snout is slightly concave to straight along its margins, its length 1.3 to 1.4 in the head or the distance from eye to snout tip equal to distance from eye to posterior angle of pectoral; length from eye to tip of snout 3.3 to 3.6

times the distance between the spiracles; its length from the mouth is 2.6 to 3.2 times the width of the mouth. The narrow rostral ridges are very close together for more than half their length, diverging posteriorly. Diameter of eye about 9 times in the preorbital length of snout in the young, and 12 to more than 13 in adults. Spiracles behind and very near eyes and not much smaller, the folds little developed or the inner one absent. Nostrils oblique, twice or nearly twice in the slightly curved mouth. First dorsal about twice as high as the length of its base, its origin more or less twice as far behind the ventrals as the distance between the two dorsals, which are subequal in size.

Skin rough to the touch with a row of 15 to 30 large strong spines along the middle of the back from the head to the first dorsal; there may be one or more between the dorsals; rows of smaller spines occur on the shoulders, around the eyes and spiracles, and along both rostral ridges. With age the spines become blunt and less prominent.

The color is more or less reddish brown above, the snout often much paler with translucent margins, the dorsals usually gray; the under side is white or whitish.

This large guitarfish reaches a length of two and a third meters or more, though I have seen no Philippine specimens more than one and three-fourths meters in length. It occurs generally throughout the Philippines, especially among the islands and reefs of the Sulu and China Seas. It lives on flats which may have no more than a meter or two of water at low tide, down to at least 65 fathoms. One was taken by trawl at this last named depth in the China Sea south of Bataan Peninsula. It is seen in the Cebu market, on the drying mats of Sulu Province, and is taken at the mouth of Malampaya river and in Malampaya Sound; it is frequently caught on the flat sandy shores of the region about Sandakan, Borneo. It is to be expected in the Gulf of Lingayen.

Its range extends to the coast of China and throughout the East Indies to Australia, Indo-China, Malaya, India, Ceylon, the Red Sea, and the east coast of Africa.

RHINOBATOS OBTUSUS Müller and Henle. **The triangle-snout guitarfish.**

Rhinobatus (*Rhinobatus*) *obtusus* MÜLLER and HENLE, Plagiost. (1841) 122, *pl. 37, fig. 2*, India; Pondicherry; Malabar; DUMÉRIL, Hist. Nat. Elasmobr. (1865) 493, Malabar; Indies; Pondicherry.

Rhinobatus obtusus GÜNTHER, Cat. Fishes 8 (1870) 443, East Indies; NORMAN, Zool. Soc. London Proc. 62 (1926) 950, *text fig. 4*, India; East Indies.

Rhinobatus obtusus FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 317, after Norman.

Rhinobatus halavi DAY (not of Forskål), Fishes India (1878) 731, *plate 193* (not 192 as given by Norman and Fowler), *fig. 4*, Mangalore.

The short broad bluntly pointed snout forms an equal sided triangle, the distance from the origin of the pectoral to the tip of the snout being equal to the distance across the base of the snout in a specimen over two meters long; margins of the snout slightly concave in same specimen; rostral ridges diverging anteriorly and posteriorly and well separated throughout, Eye about 7.3 times in the preorbital length of the snout; Norman gives 8 for specimens less than a meter in length. Only the outer fold of the spiracle is developed; spiracles about as large as eyes, and immediately behind them. Nostrils rather long, oblique, their length twice in that of the mouth and nearly equal to the distance between their inner ends. The narrow high and pointed first dorsal is about twice as high as its basal length. The origin of the first dorsal is twice or more than twice as far behind the base of the ventrals as the distance between the two dorsals; the second dorsal a little lower than the first. The rather large caudal is lunate with narrow lobes.

There is a row of denticles or tubercles down the middle of the back, with smaller denticles on top of the head and on the shoulders. The spinal row is continued between the two dorsals. Some of those above the backbone may be spinous, but with age they become flattened and the body may be nearly smooth.

The color is gray to reddish gray or brownish gray above, the dorsals and caudal paler, the under side whitish to white. The head, disk, and trunk back to the second dorsal are sprinkled with small white or whitish spots, which may be arranged in 4 or 5 longitudinal rows along each side of the body, and may also be in rows on the pectorals.

A specimen over two meters long was taken by otter trawl off the east coast of Panay, near Guimaras Island. This is the only positive Philippine record to date but no doubt it is of general occurrence from Luzon southward. It is known

from the East Indies and the shores of India and southward in the Indian Ocean to Natal.

RHINOBATOS THOUIN (Anonymous).

Raja thouin Anonymous, Allgem. Lit.-Zeit. 3 (1798) 287, 677, 685, *plate 1, figs. 3–4.*

Raia thouiniana SHAW, General Zool. 2 (1804) 318, *plate 147, fig. 2.* On *La raie thouin* LACÉPÉDE, Hist. Nat. Poiss. 1 (1798) 134, *pl. 1, figs. 3–5.*

Rhinobatus thouini van HASSELT, Algem. Konst. Letterbode (1823), Java; Sci. Nat. Ferussac Bull. 2 (1824) 90, Java; GÜNTHER, Cat. Fishes 8 (1870) 442, Penang; East Indies; WEBER, Siboga Exped., Fische 57 (1913) 597, Makassar and Aru Islands; GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 276, Penang; CHABANAUD, Econom. Indo-Chine Bull. (169) 6 (1924) 563, Gulf of Siam; SUVATTI, Index Fishes Siam (1937) 4, Canthaburi; Samut Prakan; Maenam Wen, East Coast.

Rhinobatus (*Rhinobatus*) *thouini* MÜLLER and HENLE, Plagiostomen (1841) 120; DUMÉRIL, Hist. Nat. Elasmobr. 1 (1865) 500, *plate 10, fig. 2 a–b,* Red Sea.

Rhinobatus thouinianus FOWLER, Acad. Nat. Sci. Philadelphia Proc. (1904) 460, Baram, Borneo.

Rhinobatus thouiniana NORMAN, Zool. Soc. London Proc. (1926) 951, Penang; Malay Archipelago.

Rhinobatus thouin FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 317, after Norman.

Rhinobatus (*Rhinobatus*) *ligonifer* CANTOR, Asiatic Soc. Bengal Journ. 18 (1849) 1397, *pl. 14,* Penang; Malay Peninsula; Singapore; BLEEKER, Verh. Bat. Gen. 24 (1852) 59, Plagiostomen; Bantam and Samarang.

The rather long snout has its tip distinctly enlarged so that its margins are definitely concave, its length from eye to tip 3.3 to 3.6 times the distance between the spiracles; its length from mouth to tip is 3 times the width of the mouth. Rostral cartilege narrow, wider anteriorly, the ridges close together most of their length. The eye is 7 to 8.75 times in the snout, measured from tip to eye. Both folds of the spiracle are poorly developed, the outer one more prominent. The oblique nostrils are nearly twice as long as the space between them, and are about 1.3 times in the width of the mouth; the anterior valve does not extend inwards. First dorsal about twice as high as the length of its base; its origin is 1.2 to 1.25 times as far behind the base of the ventrals as the distance between the dorsals; second dorsal nearly equal to the first; caudal long, narrow, and pointed.

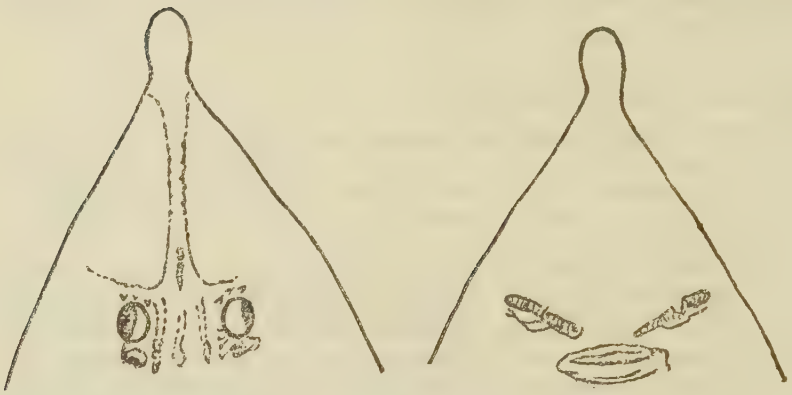

FIG. 1. Characteristic outline of snout of *Rhinobatos thouin.*

Left—upper surface
Right—lower surface
(After Norman)

There are enlarged denticles or tubercles above the spine and smaller ones in front of each eye, above the spiracles; two on each shoulder, the inner one the larger.

The color is light rusty brown, the dorsals and caudal fin darker; the under surface varies from little paler to yellowish white.

The peculiar widened tip of the snout separates this guitarfish at once from all others. I have no specimens from the Philippines, but have observed it on the drying racks at Sitankai. It is common all along the northern coast of Borneo and therefore occurs in Philippine waters from Sulu Province to Balabac. It is common in the fish market at Kuching, Sarawak, where I purchased several. It is said to reach a length of more than 1.75 meters, but I have seen none more than a meter long. It is rather abundant on the coast of Malaya, and ranges from Penang and Siam through the East Indies to New Guinea.

RHINOBATOS ARMATUS Gray. **The armed guitarfish.**

? *Rhinobatus typus* BENNETT, Life of Raffles (1830) 694, Sumatra. *Rhinobatus armatus* GRAY, Illus. Indian Zool. 2 (1834) *pl. 99;* PETERS, Monatsber. Wiss. Akad. Berlin (1877) 853, Bougainville Island, Solomon Islands; OGILBY, Queensland Mus. Mem. 5 (1916) 85, *fig. 1;* NORMAN, Zool. Soc. London Proc. (1926) 952, *text fig. 6,* India; Singapore; Makassar; Sumatra; Cape York; North Australia; Groote Eylandt, Northwest Territory; Western Aus-

tralia; HERRE, Pan Pacific Research Inst. Journ. 8 (1933) 2, Sandakan, Brit. North Borneo; HERRE, Fishes 1931 Philip. Exped. (1934) 12, Sitankai, Sulu Province.

Rhinobatus (*Rhinobatus*) *armatus* MÜLLER and HENLE, Plagiostomen (1841) 119, India; BLEEKER, Verh. Bat. Gen. 23 (1850) 13, Java; BLEEKER, Nat. Tijds. Ned.-Ind. 3 (1852) 85, Singapore; BLEEKER, Acta Soc. Sci. Indo-Neerl. 1 (1856) 10, Makassar, Celebes; BLEEKER, *ibid.* 6 (1859) 3, Doreh, New Guinea; DUMERIL, Hist. Nat. Elasmobr. 1 (1865) 494, *pl. 10, fig. 4* (scutes).

Rhinobatus granulatus GÜNTHER, Cat. Fishes 8 (1870) 443, in part; not of Cuvier; DAY, Fishes India (1878) 732.

Rhinobatus halavi GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 275, in part, exclud. synonymy; Southern Celebes and Singapore.

Rhinobatus armatus HERRE, 1936–37 Exped.; Sandakan, North Borneo.

Rhinobatus typus FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 319, after Norman.

This large guitarfish reaches a length of more than two meters, though Norman's largest specimen was but 550 mm long. Specimens up to a meter and a half long are taken in the Sibutu Islands.

The head is pointed, the snout bluntly rounded, its margin slightly concave. From the eye to the tip of the snout is 3 to 3.2 times the distance between the spiracles, and from the mouth to the snout tip is 2.5 times the width of the mouth. The rostral ridges are close together, only diverging a little posteriorly. The eye is 6.2 to 8.2 in the preorbital length of the snout, with age the eye becoming proportionally smaller. The large spiracles are close behind the eyes, each with two feeble folds on its rear margin, the outer one more prominent. The large oblique nostrils are five-sixths of or nearly equal to the width of the mouth, and 2.5 to 3 times as long as the space between their lower or inner ends. The first dorsal is twice or more than twice as high as the length of its base; the distance of its origin behind the base of the ventrals is 1.3 to 1.6 times the distance between the two dorsals.

There is a row of rather strong compressed spines along the middle of the back, and one or two groups over each shoulder; there are usually several smaller spines around each eye and spiracle. The denticles on top of the head, on the back, and on the shoulders are larger than elsewhere.

The color above is gray, shading a yellowish or reddish, but often the upper side is uniformly grayish brown; the lower surface is white, with a brown spot near the tip of the snout.

The armed guitarfish is common on the extensive reefs and flats about Sitankai and the other Sibutu Islands, Sandakan, and ali along the north coast of Borneo. It is often seen on the drying platforms at Sitankai. No doubt it occurs also on the shores of Mindanao, Palawan, and the Visayan Islands. It ranges from the shores of India and Malaya through the East Indies to the Solomon Islands and tropical Australia.

RHINOBATOS HALAVI (Forskål).

Raja halavi FORSKÅL, Descrip. Anim. (1775) 19.

Rhinobatus halavi RUPPELL, Atlas, Fische (1828) 55, *plate 14, fig. 2;* GÜNTHER, Cat. Fishes 8 (1870) 442, in part; KLUNZINGER, Verh. zool.-bot. Gesells. Wien 21 (1871) (Synopsis Fische Roth. Meeres, part II, 235); NORMAN, Zool. Soc. London Proc. (1926) 956, *text fig. 9.*

Rhinobatus (*Rhinobatus*) *halavi* MÜLLER and HENLE, Plagiostomen (1841) 120; DUMERIL, Hist. Nat. Elasmobr. 1 (1865) 496.

Rhinobatos halavi FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 320.

Rhinobatus granulatus BAMBER, Linn. Society London Journ. Zool. 31 (1915) 477, not of Cuvier.

The nearly triangular snout is of moderate length, with bluntly rounded tip, the nearly straight margins slightly concave; the preorbital length is about 1.35 times in its width and 2.75 to 3 times the distance between the spiracles; the length from mouth to tip of snout is 2.6 to 2.66 times the width of the mouth. The narrow rostral ridges are close together most of their length, diverging a little anteriorly and posteriorly. The eye is contained 5 to 6 times in the preorbital length of the snout; spiracles deep but much smaller than the eyes, and close to them, with two folds on each rear margin, the inner one small. Nostrils oblique, medium in size, their length about 1.5 times in the width of the mouth. The first dorsal is twice as high as its basal length; the distance of its origin from the base of the ventrals is 1.66 to nearly twice in the space between the two dorsals.

The skin is smoother than in most guitarfishes but the denticles on top of the head, shoulders, and back are enlarged; a row of 15 to 20 spines from the head to the first dorsal and a single (2 ?) spine between the dorsal fins. Usually there are 2 or 3 large spines on the ridge before the eye and above each spiracle, and one or two spines on each shoulder. Young specimens have a row of spines along each ridge of the snout. With age all spines tend to become smooth or to disappear.

The color is olive brown above, becoming more or less reddish brown along the margins of the head and disk; under surface white or whitish.

The recorded length is 1,500 mm, and it probably reaches 2 meters.

No specimens have been seen from Philippine waters, but it was recorded in 1895 by Casto de Elera from the Gulf of Lingayen, La Union Province. It may very well occur there and indeed all along the western coast of the Philippines from Balabac to Ilocos Norte. It should be looked for carefully on the west coast of Palawan, especially in the fish corrals of Malampaya Sound, and in the trawler catches of the Gulf of Lingayen.

Its known range is from the Red Sea all along the coast of Asia to China. It has also been reported from Australia.

RHINOBATOS FORMOSENSIS Norman. The Formosan guitarfish.

Rhinobatos formosensis NORMAN, Zool. Soc. London Proc. 62 (1926) 958, *text fig. 11*, type locality, Formosa; FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 307, after Norman; HERRE, Check List Philip. Fishes (1953) 37.

The long bluntly pointed snout is rather narrow, its pre-orbital length almost equal to its width at the upper margin of the eyes; the preoral length is 3.5 to 3.66 times the width of the mouth. The eye is 4.3 to 4.6 times in the pre-orbital length of the snout. The spiracles are much smaller than the eyes and immediately behind them; the outer fold of the spiracle is well developed, the inner one small. The narrow rostral ridges are separated more or less almost their whole length, converging and very close together at their tip, diverging posteriorly. The oblique nostrils are of moderate size, their length 1.4 to 1.5 times in the width of the mouth, and about 1.5 times as long as the space between their inner ends.

The height of the first dorsal is less than twice its basal length; its origin is behind the ventral base a distance equal to or greater than the space between the two dorsals.

The skin is smooth to the touch, its denticles minute. There is a row of rudimentary tubercles along the midline of the back, around the eyes, and the spiracles, so small as to be scarcely visible to the naked eye.

This guitarfish was described by Norman from two specimens from Formosa, 625 and 630 mm in length. A larger

specimen almost a meter in length, was determined by the well-known Filipino ichthyologist, A. F. Umali. It was taken by otter trawl in the China Sea off the south entrance to Manila Bay, at a depth of 65 fathoms, or over 118 meters. No doubt it occurs in the deeper parts of Manila Bay, near Corregidor, and all along the western coast of Luzon, particularly in the Gulf of Lingayen.

RHINOBATOS SCHLEGELI Müller and Henle.

Rhinobatus (*Rhinobatus*) *schlegeli* MÜLLER and HENLE, Plagiostomen (1841) 123, *plate 42*, Nagasaki.

Rhinobatus schlegelii RICHARDSON, Ichthy. China, Japan (1846) 195; GÜNTHER, Cat. Fishes 8 (1870) 445, Japan but not Formosan specimen; ELERA, Cat. Fauna Filip. 1 (1895) 619, Navotas, Manila, Luzon; a dubious reference; STEINDACHNER, Hofmuseum Wien. Ann. 11 (1896) 225, Kobe, Hiogo, Nagasaki; GARMAN, Mus. Comp. Zool. Mem. 36 (1913) 276, Japan; NORMAN, Zool. Soc. London Proc. 62 (1926) 959, *fig. 12*, Japan.

Rhinobatus schlegeli SCHLEGEL, Fauna Japonica, Poiss. (1950) 307, 1848, Nagasaki Bay; JORDAN and SNYDER, U. S. Nat. Mus. Proc. 23 (1900) 337, Tokyo; JORDAN and EVERMANN, U. S. Nat. Mus. Proc. 25 (1902) 318, Formosa; TANAKA, Jap. Fish. Life Colors 29 (1933).

Rhinobatus schlegel JORDAN and FOWLER, U. S. Nat. Mus. Proc. 26 (1903) 645, Tokyo Wakanoura, Onomichi, Hakata, Nagasaki.

Rhinobatos schlegeli MORI, Pan. Pacif. Research Ins. Journ. 3 (1928) 3, Fusan and Mokpo, Korea; FANG and WANG, Contrib. Biolog. Lab. Sci. Soc. China 8 (1932) 259, *fig. 19*, Tsingtau, China.

Rhinobatos schlegeli FOWLER, U. S. Nat. Mus. Bull. 100 13 (1941) 308, Simoda, Wakanoura, Hakata, Japan.

The snout is long, with bluntly rounded tip and concave margins; the rather narrow parallel rostral ridges are very close together anteriorly, diverging a little posteriorly. The eye is 4.85 to nearly 5 times in the preorbital length of the snout; the small spiracles are very close to the eyes, their folds little developed, the outer one more evident than the inner. The short nostrils are rather broad, 1.6 times in the width of the mouth, and 1.3 to 1.4 times more than the space between their inner ends. The height of the pointed first dorsal is about twice the length of its base. The origin of the first dorsal is a little farther from the ventral base than the distance between the two dorsals.

The skin is smooth to the touch, though covered with minute denticles. A row of about 3 dozen small blunt tubercles or spines extends down the middle of the back; small spines are

present or absent around the eyes and above the spiracles; none are on the shoulders.

The color is brown above, mottled or speckled with darker, the sides of the snout translucent or paler brown. The under surface is whitish, except for a black or dusky blotch under the tip of the snout. The young may have many widely scattered small rounded whitish spots over the disk and trunk.

For a long time this guitarfish, which reaches a length of about a meter, was only known from Japan, but it also occurs on the shores of Korea, China, and Formosa. It was reported from Manila Bay by Casto de Elera but his determinations are often too doubtful to be accepted without later confirmation. However in view of its distribution it may well be in Philippine waters. It should be looked for in the Gulf of Lingayen and the north coast of Cagayan Province, Luzon, as those waters contain many Formosan fishes.

ILLUSTRATIONS

Plate 1

Fig. 1. The white-spotted guitarfish, known as *pating sodsod, Rhynchobatus djiddensis* (Forskål).

2. Rough-backed guitarfish *Rhinobatus granulatus* Cuvier.

Plate 2

Fig. 3. A fine specimen of *pating sodsod* over two meters long, from the China Sea, off Cavite Province.

4. A round-snout guitarfish (*Rhina ancylostoma* Bl. and Schn.) over two meters long. Caught near entrance to Manila Bay.

Plate 3

Fig. 5. The triangle-snout guitarfish (*Rhinobatus obtusus*) over two meters long, from the east coast of Panay.

6. A specimen of *Rhinobatus obtusus* from the east coast of Panay. It was over two meters long.

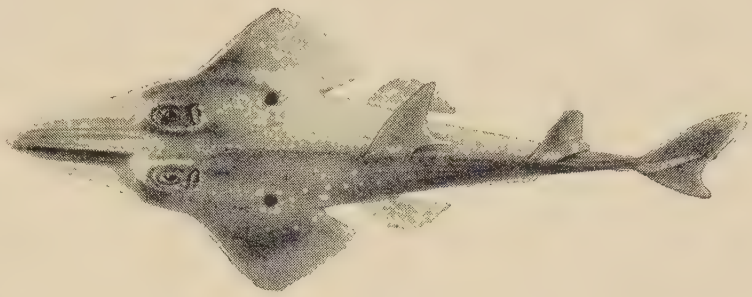

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PLATE 1.

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PLATE 2.

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PLATE 3.

JAPANESE PSYCHODIDÆ, II

NINE NEW SPECIES OF MOTH FLIES *

By Maasaki Tokunaga and Etsuko Komyo
Entomological Laboratory, Saikyo University, Kyoto, Japan

THIRTY-ONE TEXT FIGURES

In the preceding paper ** we reported ten Japanese *Psychoda* species, including four new species. In this paper we present other nine Japanese species, including one of *Sycorax* Haliday (Trichomyiinæ), two species of *Trichopsychoda* Tonnoir, two species of *Brunettia* Annandale and four species of *Pericoma* Walker (latter three genera, Psychodinæ).

Abbreviation.—TTR is tibiotarsal ratio, relative length of tibia to that of first tarsal segment of leg. TR is tarsal ratio, relative length of first tarsal segment to that of second tarsal segment of leg. RR is radial ratio, relative length of wing vein R_{2+3} to that of R_2. The morphological terminology used in this paper is mainly adopted from that of Dr. P. Freeman (1950).

We express our hearty thanks to Dr. T. Ishihara and Mr. T. Yano, of Matsuyama College of Agriculture; Mr. Y. Shogaki, of Kyoto; and Mr. M. Sasakawa, of our university, for their collection of the moth flies which are rather difficult to capture.

PERICOMA SHIKOKUENSIS Tokunaga et Etsuko, sp. nov.

This is a pale brown species collected at Mt. Saragamine, Shikoku. Wings show mottled appearance being provided with dark tufts of hairlike scales at the tips of longitudinal veins, at the bases of R_2 and M_{1+2}, on R_{4+5} and M_{3+4} and at middle of M_3 and pale tufts of fringe at tip of wing, and at tips of R_1, R_2 and other longitudinal veins. Legs are provided with paler rings on the basal one-third of first and second tarsal segments of all legs.

Male.—Body length about 1.8 mm.

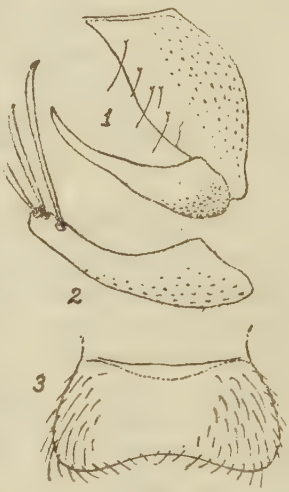
Eyes separated above as wide as three facets. Relative lengths of palpal segments about 25:36:44:50. Antennæ 16-segmented; basal two segments subequal in length; flagellar

* Contribution from the Entomological Laboratory, Saikyo University, No. 34.

** Philip. Jour. Sci. 83 (1954) 307–317.

segments elongated oval, but 14th and 15th short and subspherical; ultimate with an apical papilliform projection. Ascoids of flagellar segments simple and curved. Relative lengths of distal eight antennal segments about 14:13:12:11:11:9:9:13.

TTR and TR of hind leg about 2.13 and 2.67 respectively. Wings comparatively narrow, with tip round, membrane faintly brown, veins slender and pale brown. Venation: R_{2+3}, R_5, M_2 and Cu_1 somewhat obscure at base, fork of R_{2+3} slightly beyond that of M_{1+2} which is at level of end of Cu_1, RR about 0.57, R_4 ending just before wing tip, R_{2+3+4} not longer than twice of width of anterior basal cell, fork of M_{3+4} not distinctly petiolate, Cu_1 distinctly separated basally from fork of M_{3+4}.



Pericoma shikokuensis Tokunaga et Etsuko, sp. nov.

FIG. 1. Male, coxite and style, dorsal aspect.
2. Male, cercus, lateral aspect.
3. Female, lobes of subgenital place.

Hypopygium (Figs. 1 and 2) with coxites somewhat elongated; styles claw-like, slender almost as long as coxites, sharply pointed, slightly arcuate, but base swollen and scaly on lateral side; cerci stout, arcuate, not distinctly swollen basally, each with three large simple retinacula on apical part.

Female.—Body about 2.34 mm, far larger than male; coloration similar to that of male.

Eyes widely separated above, as wide as three and a half facets. Antennæ almost as in male; relative lengths of distal eight segments about 15:14:13.5:13:13:9:10:13. Legs with first and second tarsal segments narrowly pale at both ends; TTR and TR of hind leg about 2.29 and 2.82 respectively. Wings 2.12 × 0.8 mm; venation: R_{2+3} and R_5 somewhat obscure at base, fork of R_{2+3} just beyond that of M_{1+2}; RR about 0.5. Ovipositor normal; subgenital plate (Fig. 3) with caudal lobe transverse, not subdivided, very slightly concave on caudal margin, its lateral angles obtuse.

Locality.—Shikoku, Japan.

Holotype.—Male; Mt. Saragamine, Ehime-ken, Shikoku; May 17, 1953 (*Mr. T. Yano*).

Allotopotype.—Female; May 17, 1953 (*Mr. T. Yano*).

Type specimens.—Mounted in Gater's solution, deposited in the entomological laboratory of Saikyo University.

This species is somewhat allied to *P. blandula* Eaton and *P. trifasciata* Meigen. However in the first allied species, the wings are relatively broader and vein R_{2+3} is comparatively longer than in the new species, and the male hypopygium differs in possession of five retinacula at tip of cercus differing from the present species. In the second allied species, following points are distinctly different from the present species: the fore tibiæ are entirely white usually and the male hypopygium is provided with short coxites which are shorter than styles, styles are relatively longer and distinctly more swollen basally and cerci are provided with four retinacula on each distal end. The species name is related to the locality of the present moth fly.

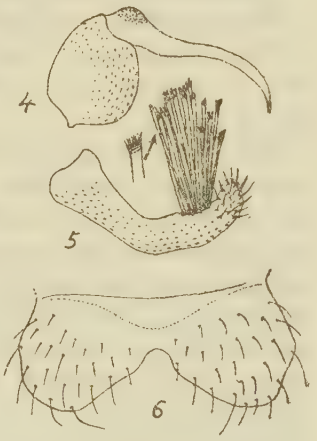
PERICOMA YANOI Tokunaga et Etsuko, sp. nov.

This is a large black species. The wings are entirely black and the legs are also black but provided with minute pale rings at distal tips of tibiæ, first and second tarsal segments.

Male.—Body, about 3 to 4 mm long.

Eyes separated above as wide as three facets. Maxillary palpi 4-segmented (30:61:65:58). Antennæ with first segment relatively long, about twice of width and second subspherical, about a half as long as first; flagella broken off.

TTR and TR of hind leg about 2.51 and 2.4 respectively. Wings about 4 mm long, about thrice the width, with membrane dark brown, veins dark and wing tip round. Venation: Sc short, a veinlike fold extending from the tip of Sc along the axis of cell between costa and R_1, fork of R_{2+3} and that of M_{1+2} subopposite to each other and far before tip of Cu_1, R_5 ending slightly beyond tip of wing, fork of R_{2+3} and base of M_2 slightly thickened, Cu_1 obscurely connected with M_{3+4} which is slightly longer than R_{4+5}.



Pericoma yanoi Tokunaga et Etsuko, sp. nov.

FIG. 4. Male, coxite and style, lateral aspect.

5. Male, cercus, lateral aspect.

6. Female, lobes of subgenital plate.

Hypopygium (Figs. 4 and 5) massive; coxities very short, far shorter than styles and bulbous; styles slender, as long as cerci, pointed, undulated, and at base slightly swollen dorsally and scaly; cerci stout, far longer than ninth tergite, angulated, with tip round and crowned with many spinules; retinacula long, pre-apical in position and thirteen in number.

Female.—Similar to male, with usual sexual differences. Antennæ 16-segmented; first segment about twice as long as second; relative lengths of distal eight segments about 12:11:-11:11:11:8:8:10. TTR and TR of hind leg about 2.76 and 2.27 respectively. Wings with forks of R_{2+3} and M_{1+2} slightly before tip of Cu_1, RR about 0.48, and R_{2+3} far shorter than M_{1+2} (17:23). Subgenital plate (Fig. 6) with two lobes which are subdivided by a wide V-shaped caudal incision.

Locality.—Shikoku, Japan.

Holotype.—Male; Matsuyama, Ehime-ken; April 10, 1952.

Allotopotype.—Female; April 10, 1952.

Paratopotypes.—A male and a female; April 10, 1952.

Type-specimens.—Mounted in Gater's solution; deposited in the entomological laboratory of Saikyo University; collected by Mr. T. Yano.

This new species resembles to *P. avicularia* Tonnoir, especially in the coloration. But these two species are quite different in the structure of the hypopygium. In the allied species, differing from the present species, the styles of the male are strongly swollen basally and cerci are provided with five short, retinacula at each tip and the caudal lobe of the female subgenital plate is somewhat square and shallowly incised at caudal margin and separated into two blunt lobes. This new species is named in honor of the collector, Mr. T. Yano, of Matsuyama College of Agriculture.

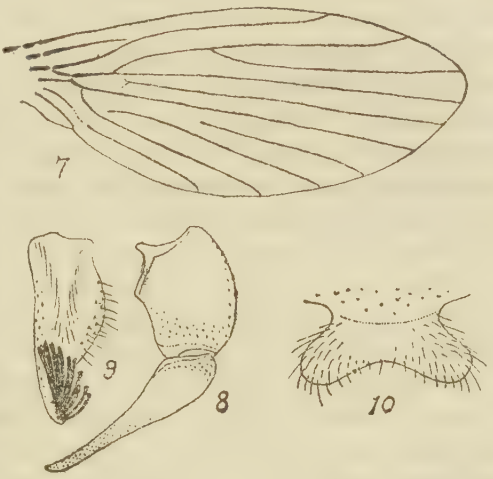
PERICOMA SASAKAWAI Tokunaga et Etsuko, sp. nov.

This is a black species, with a white tuft of fringe on the apical part of the wing and black tufts of hairs on tips of longitudinal veins and several obscure pale tufts of hairs on main veins. Coloration of legs is sexually different.

Male.—Body almost entirely black. Wing with whitish pale brown apical fringe between tips of R_3 and R_5 and obscure small tufts of whitish hairs on following veins: each one tuft on R_1 beyond middle, on R_2 and R_3 before middle, on R_4 beyond middle, on M_2 at middle, on M_3 before tip, on M_4 at middle,

and on Cu_1 before tip. In addition to these pale patterns, small black tufts of hairs at tips of main veins, at fork of R_{2+3} and at base of M_2. Legs black, but distal two tarsal segments of all legs paler.

Eyes separated above as wide as three facets. Maxillary palpi 4-segmented (21 :33: 35: 44). Antennæ 16-segmented; first segment about twice as long as second which is subspherical; first flagellar segment as long as preceding segment, with characteristic lateral flat lobe which is slightly extended distad and bears three rarely two, large bristles on lateral ridge; other flagellar segments comparatively short, cask-shaped; ultimate segment somewhat elongated, with a papilliform distal projection; ascoids straight; relative lengths of distal eight segments about 9:8.5:8:8.8:7:7:8.



Pericoma sasakawai Tokunaga et Etsuko, sp. nov.

FIG. 7. Male, wing, hairs and scales omitted.
8. Male, coxite and style, dorsal aspect.
9. Male, cercus, dorsal aspect.
10. Female, lobes of subgenital plate.

Thorax black. Legs black, but ultimate two tarsal segments paler. TTR and TR of hind leg about 3.0 and 2.3 respectively. Wings 2.5 × 0.9 mm; membrane dark brown; costal cell darker; veins brownish black. Venation (Fig. 7) : base of R_{2+3} slightly before that of R_5, RR about 0.6, R_4 ending just before tip of wing, M_2 obscure at base, Cu_1 separated from fork of M_{3+4} and its base slightly before level of fork of M_{3+4}.

Abdomen black. Hypopygium (Figs. 8 and 9) with coxites short and subspherical; styles straight and longer than coxites; cerci with 11 to 12 retinacula on each distal part.

Female.—Body black; legs and wings with pale pattern. Wings with pale brown apical fringe and marginal black tufts of hairs at ends of veins as in male, but with paler smaller

tufts of hairs at basal part of costal margin, on apical parts of R_2, R_3 and R_4, on basal part of M_1 and before tip of M_3. Legs with knee-joints, distal ends of tibiæ and first tarsal segments and entire second tarsal segments whitish pale brown. Other paler hairs present on vertex of head, on dorsal side of abdominal base and on lateral sides of thoracic notum above wing bases.

Eyes separated above as wide as three facets. Maxillary palpi 4-segmented (25:35:37:46). Antennæ 16-segmented; first segment cylindrical; second segment elongated oval, about two-thirds as long as first; flagellar segments of basal part subspherical to elongated oval, those of distal part fusiform; relative lengths of distal eight segments about 12:12:13:12:-10:7.5:6.5:8.5; ascoids almost straight.

TTR and TR of hind leg about 2.37 and 2.1 respectively. Wings 3.3 × 0.9 mm, somewhat pointed at tip; membrane pale brown; veins brown, stout, especially R_1, R_{2+3}, R_2, M_4 and Cu_1 strong; venation: base of R_{2+3} far before fork of R_{4+5} and R_{4+5} nearly thrice of width of anterior basal cell (14:5), RR about 0.65, R_4 ending just before wing tip, M_2 somewhat thickened at base, fork of M_{1+2} at level of end of Cu_1 or just before it and distinctly before fork of R_{2+3}, M_3 obscure at base, Cu_1 separated from fork of M_{3+4} and atrophied at base.

Ovipositor normal; lobe of subgenital plate (Fig. 10) constricted at base, its caudal margin evenly concave and its lateral projections divergent and round.

Locality.—Honshu, Japan.

Holotype.—Male mounted in Gater's solution; Kibune, Kyoto; May 17, 1954 (*Mr. M. Sasakawa*).

Allotopotype.—Female mounted in Gater's solution; Midoro-ike, Kyoto; May 9, 1953 (*Mr. Y. Shogaki*, at light).

Paratopotypes.—Two dry males; May 17, 1954 (*Mr. M. Sasakawa*).

Type-specimens.—Deposited in the entomological laboratory, Saikyo University.

The present new species is somewhat allied to *P. avicularia* Tonnoir in the coloration, but the male hypopygium is quite different. In the allied species, the coxites are slender and almost as long as the styles and the styles are swollen basally. The wings of the allied species are provided with paler tufts of hairs at tips of most longitudinal veins. The male hypopygium of the new species more resembles to that of *P. cana-*

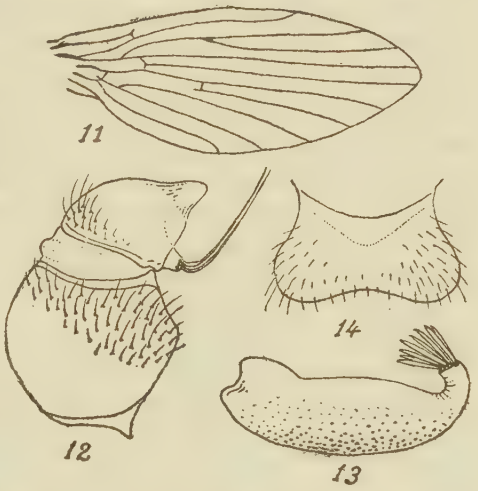
riensis Tonnoir, but in the allied species the male antennæ normal without the special lobe of the first flagellar segment differing from the new species. The present species is named in honor of Mr. M. Sasakawa who kindly collected this peculiar moth fly.

PERICOMA OMOGOENSIS Tokunaga et Etsuko, sp. nov.

This is a large brown species, with mottled wings and dark tufts at tips of most veins, forming a characteristic dotted border.

Male.—Head, thorax and abdomen dark brown. Wings pale, with many dark tufts of hairs at tips of all longitudinal veins, at forks of R_{2+3} and M_{1+2}, at base of R_{s+z} and M_{1+2}, on distal margin of anterior basal cell and on coastal margin before tip of R_1. Tibiæ and first tarsal segments of all legs white and their distal ends narrowly dark; femora brown; four distal tarsal segments dark.

Head with eyes separated above as wide as three facets. Maxillary palpi 4-segmented (19:30:40:64). Antennæ 16-segmented; first segment cylindrical; second subspherical; flagellar segments fusiform and elongated; ascoids slightly curved; relative lengths of basal four and distal eight antennal segments about 25:18:17:13 and 12:12:12:12:12:10:9:11.5 respectively.



Pericoma omogœnsis Tokunaga et Etsuko, sp. nov.

FIG. 11. Male, wing, hairs and scales omitted.
12. Male, coxite and style, dorsal aspect.
13. Male, cercus, lateral aspect.
14. Female, lobes of subgenital plate.

Hind legs with TTR and TR as follows: 1.96 and 3.0 respectively. Wings (Fig. 11) elongated oval, round at tip, 2.9 × 1.1 mm, with membrane almost hyaline, veins pale brown. Venation: RR about 5.7, R_{2+3} arised far before fork of R_{4+5}, R_{4+5} about twice as long as width of anterior basal cell, R_5 ending

just beyond wing tip, M_2 with a short spur-like vein at base, forks of R_{2+3} and M_{1+2} opposite to each other and far before tip of Cu_1, Cu_1 not connected to fork of M_{3+4} and its base before level of the fork.

Hypopygium (Figs. 12 and 13) with coxites short, globular, as long as width; styles very short, conical and with apical papilliform projection in dorsal aspect, latero-ventral edge produced ventrad forming a triangular projection, from which two or three long curved bristles arise; cerci large and strong, tapering and curved dorso-cephalad at tip, with eight short but strong apical retinacula.

Female.—Almost as in male except for usual sexual differences.

Head with eyes widely separated above as wide as four facets. Maxillary palpi 4-segmented (26:35:41:60). Relative lengths of basal four and apical eight antennal segments as follows: 21:17:14:12 and 12:12:12:12:11.5:9:8:11. TTR and TR of hind leg about 2.86 and 2.44 respectively. Wings 2.16 × 1.23 mm; RR 0.64; R_{4+5} a little longer than width of anterior basal cell (8:6). Lobe of subgenital plate (Fig. 14) somewhat trapezoid, distinctly constricted at base, slightly concave at caudal margin, its latero-caudal ends round.

Locality.—Shikoku, Japan.

Holotype.—Male; Omogokei, Ehimeken; May 26, 1953.

Allotopotype.—Female; May 26, 1953.

Paratopotype.—Male; May 26, 1953.

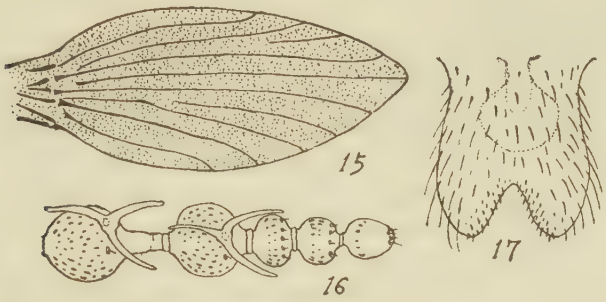
Type specimens.—Mounted in Gater's solution; collected by Mr. T. Yano; deposited in the entomological laboratory, Saikyo University.

This new species is closely allied to *P. canescens* Meigen in the coloration and venation of the wings but distinctly differs from the allied species in the structure of the male hypopygium. The coxites of the allied species are longer, being fully twice as long as the width and the styles are longer than the coxites, elongated and clawlike. The species name is related to the locality of this moth fly.

TRICHOPSYCHODA SHOGAKII Tokunaga et Etsuko, sp. nov.

The wings of this species are covered with hairlike scales on entire membrane and veins R_3 and M_2 are atrophied at bases

Female.—Body dark gray; wings with forks of R_{2+3} and M_{1+2} incomplete; antennæ 16-segmented, distal four sgements separated, of which ultimate three diminutive and subequal in size. Subgenital lobe longer than width and with V-shaped caudal incision.



Trichopsychoda shogakii Tokunaga et Etsuko, sp. nov.
FIG. 15. Female, wing, hairs and scales omitted.
16. Female, distal five antennal segments, verticil omitted.
17. Female, lobes of subgenital plate.

Head with eyes very narrowly separated above as wide as one-fifth of a facet. Maxillary palpi 4-segmented (20:23:31:34). Antennæ (Fig. 15) 16-segmented; first segment as long as second subspherical segment; flagellar segments with short neck part which is far shorter than basal swollen part; ascoids Y-shaped, short, present on basal 11 flagellar segments; distal four distinctly separated from each other; 13th antennal segment subspherical. without neck part, with ascoids and verticils as in preceding segments; 14th and 15th subequal, slightly shorter than width, with verticils but without ascoids; 16th oval, with several verticils and several apical small hairs; proportional lengths of distal eight segments about 18:15:15:14:9:5:5:6.

Hind leg with TTR and TR about 1.86 and 3.82. Wings (Fig. 15) 1.67 × 0.7 mm, pointed at tip, where vein R_5 ends, covered with hairlike scales on membrane; venation as in *Psychoda malleola* Tokunaga et Etsuko, two forks being incomplete, R_3 very short and its base beyond tip of R_1; M_2 long and about 2.56 times as long as R_3, bases of R_{2+3} and R_5 located at same level and not distinctly connected to R_4, Cu_1 connected to M_4 at point from where M_3 arises.

Ovipositor normal, not strongly curved; subgenital plate (Fig. 17) with a long caudal lobe which is slightly constricted at base, with a caudal V-shaped incision, its two caudal projection round at tip and parallel.

Male.—Unknown.

Locality.—Honshu, Japan.

Holotype.—Female mounted in Gater's solution; Midoro-ike, Kyoto; July 8, 1952.

Paratopotype.—Female mounted in Gater's solution; July 8, 1952.

Type specimens.—Collected by Mr. Shogaki by a light trap; deposited in the entomological laboratory, Saikyo University.

This species is closely allied to *T. hirtella* Tonnoir, except for the structure of the female subgenital lobe. In the allied species the subgenital lobe is deeply constricted at base and shallowly concave at caudal margin showing that the two caudal projections are divergent from each other. The paratopotype is somewhat abnormal differing from the holotype in wing venation, relative lengths of leg segments and shape of ovipositor. This species is named in honor of Mr. Y. Shogaki who collected this moth fly.

TRICHOPSYCHODA LEPIDA Tokunaga et Etsuko, sp. nov.

This species whitish gray, with wing membrane covered with scales entirely and with two forks of wing veins complete.

Female.—Body about 2 mm long, characteristic in the structures of wing veins especially in the radial field, antennal ascoids and lobe of subgenital plate.

Head round, with frontal scaly region oval, eyes narrowly separated above as wide as one and a half facets. Maxillary palpi 4-segmented (15:28:37:34). Antennæ (Fig. 18) 16-segmented, closely similar in structure to those of *T. Shogakii* Tokunaga et Etsuko, but small three distal segments covered with numerous verticils and Y-shaped ascoids of flagellar segments each with an anterior arm flattened; relative lengths of distal eight segments about 18:18:17.5:17:10:6.5:5.5:6.5.

TTR and TR of hind leg about 2.93 and 2.8 respectively. Wing (Fig. 19) 1.9 × 0.77 mm; membrane hyaline, with numerous scales; costal cell pale brown on caudal half; veins slender. Venation: anterior basal cell very short, R_5 arised far before base of R_{2+3}, entire length of R_{2+3} and R_2 taken together undulated, R_{2+3} thickened at middle, RR about 1.17, cell R_3 very narrow at middle, R_5 evenly curved and ended at

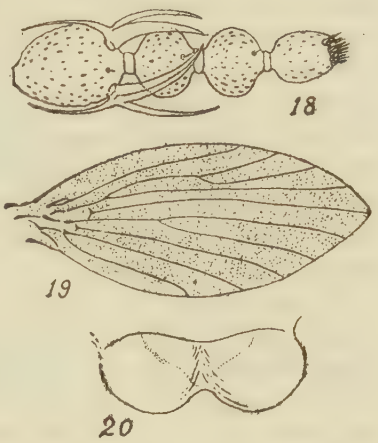
pointed wing tip, fork of M_{1+2} slightly before tip of Cu_1 and far before fork of R_{2+3}, Cu_1 connected with M_{3+4} slightly before fork of M_{3+4}.

Ovipositor normal and horny. Lobe of subgenital plate (Fig. 20) transverse, very large, with a shallow V-shaped caudal incision, its lateral half suboval.

Male.—Unknown.

Locality.—Honshu, Japan.

Holotype.—Female mounted in Gater's solution; Hachijo, Kyoto City; August, 1952 (*M. Tokunaga*); deposited in the entomological laboratory, Saikyo University.



Trichopsychoda lepida Tokunaga et Etsuko, sp. nov.

FIG. 18. Female, distal four antennal segments, verticils omitted.

19. Female, wing, hairs and scales omited.

20. Female, lobes of subgenital plate.

This new species is peculiar in possession of *Brunettia*-like wing venation, but the antennal structure is characteristic for *Trichopsychoda*. *Lepidopsychoda* Edwards shows similar wing ornamentation and antennal structure to those of the present species, but the ovipositor is not horny, differing from the present species. The male of this species is unknown yet and it may be best to refer the fly to the genus *Trichopsychoda*. The species name is related to the scales of the wings which cover the entire wing membrane.

BRUNETTIA SPINISTOMA Tokunaga et Etsuko, sp. nov.

This is a black species, with small white tufts of hairs at tips of main longitudinal veins. The wings are sexually different in shape.

Male.—Body about 1.6 mm long. Legs black, each with a small white ring at distal end of tibia and a large white band on apical half of first tarsal segment. Wings black, covered with broad scales on basal half and with slender scales on marginal half, and provided with small white tufts of hairs at tips of vein R_2, R_3, R_4, R_5, M_1, M_2, and M_3 and on costal margin before tip of R_1.

Head with a blunt vertexal projection. Eyes with narrow dorsal projections which are contiguous above with each other. Maxillary palpi 4-segmented (10:40:45:55). Labella with many short but strong spinules. Antennæ (Fig. 23) 15-segmented; first segment dilated apically and fully twice as long as second (32:15); second segment slightly discoidal; first flagellar segment flask-shaped but its neck part very short; second to 11th flagellar segments each with a short neck part; penultimate segment short and with a very short neck part; ultimate small, with a papilliform apical projection and without ascoids; ascoids large, simple, strongly curved; proportional lengths of distal eight segments as follows: 22:22:22:21:21:21:15:13.

Thorax black. TTR and TR of hind leg about 2.67 and 2.4 respectively. Wing (Fig. 21) 2 × 1.2 mm, very broad, somewhat cordate, due to the expansion of costal and anal cells, with membrane brown and veins strong, dark brown, especially in veins R_1, R_2, R_5 and M_4. Venation: longitudinal veins arranged radially; R_{2+3} arised beyond base of R_5, very short, almost as long as R_{2+3+4} (8:7), R_2 very long and 6 times of R_{2+3}, RR about 0.17, R_5 straightly extended to round tip of wing, fork of M_{1+2} slightly beyond fork of R_{2+3}, point of fork between M_3 and M_4 just under base of R_5, Cu_1 separated from M_4 and arised just under fork of M_{3+4}.

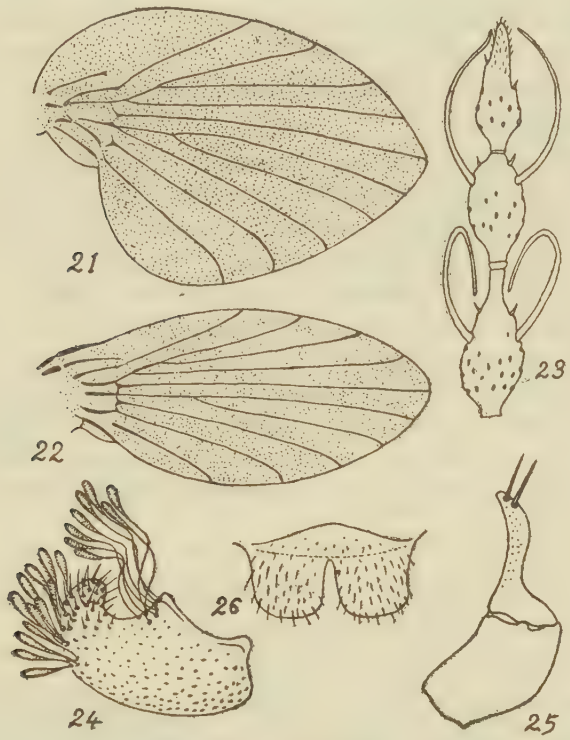
Abdomen black. Hypopygium (Figs. 24 and 25) small; coxite cylindrical, bare, slightly longer than style; style slender, slightly undulated, not sharply pointed, with two small setæ at tip; cercus small, somewhat clog-shaped, its distal part curved up-wards and blunt at tip, with many delicate retinacula on pre-apical side, longer retinacula on dorsal side at base and crowned with many short spinules at tip.

Female.—Coloration as in male. Wings normal in shape.

Eyes with narrow dorsal projections which are widely separated above from each other as wide as four facets or more. Maxillary palpi 4-segmented (10:45:44:46). Antennæ 15-segmented; flagellar segments of basal part bulbous but those of distal part slender and somewhat fusiform; penultimate segment without neck part; ultimate slender and without ascoids but with verticils. Labella of mouth parts with more distinct spinules than in male.

Hind leg with TTR about 2.33 and TR about 2.57. Wings (Fig. 22) 1.87 × 0.87 mm, normal in shape, with tip round. Venation: longitudinal veins radially arranged; R_{2+3+4} very

short, shorter than a half of R_{2+3} (3.5:9), RR about 0.21, other veins as in male.



Brunettia spinistoma Tokunaga et Etsuko, sp. nov.
FIG. 21. Male, wing, hairs and scales omitted.
22. Female, wing, hairs and scales omitted.
23. Male, distal three antennal segments, verticils omitted.
24. Male, cercus, lateral aspect.
25. Male, coxite and style, dorsal aspect.
26. Female, lobes of subgenital plate.

Subgenital plate (Fig. 26) with two caudal lobes which are separated by a deep incision from each other, about as long as width and evenly convex slightly at caudal margin.

Localities.—Honshu and Shikoku, Japan.

Holotype.—Male mounted in Gater's solution; Saga, Kyoto, July 10, 1938 (*M. Tokunaga*).

Allotopotype.—Female mounted in Gater's solution; July 10, 1938 (*M. Tokunaga*).

Paratypes.—Males and Females mounted in Gater's solution and dry; Saga, Kyoto; July 10, 1938 (*M. Tokunaga*); Kita-shirakawa, Kyoto City; November 7, 1935 (*M. Tokunaga*); Matsuyama, Ehime-ken; May 10 and July 11 and 22, 1952 (*Mr. T. Yano*).

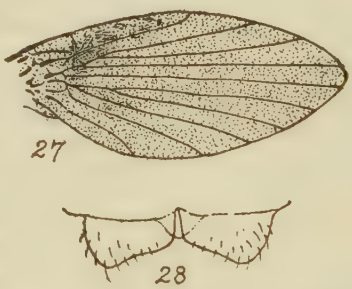
Type specimens.—Deposited in the entomological laboratory, Saikyo University.

This new species is somewhat allied to a widely distributed species, *B. albonotata* Brunett, and Samoan species, *B. biformis* Edwards. In the first allied species, the wings of both sexes are similar in shape and the female wings are provided with scales only at base differing from the new species. In the second allied species, the sexual dimorphism of the wing shape is more pronounced than in the new species and the female wings are covered with broad scales wholly differing from the present species. These three allies species are quite characteristic in the structures of the male hypopygia. This species is named in the relation to the spinulous mouth parts.

BRUNETTIA ISHIHARAI Tokunaga et Etsuko, sp. nov.

This is a black species, with yellowish and whitish tufts of hairs on the wings and white rings on the legs.

Female.—Body about 4.5 mm long. Legs dark, each with a small white ring at tip of tibia and a broad white ring on apical two-thirds of first tarsal segment or with a small dark ring on sub-basal part of white first tarsal segment. Wings dark brown, with a yellowish white apical fringe from middle of cell R_3 to middle of cell R_5, and a small white tuft of hairs at each end of R_1, R_2, R_3, M_3, M_4, and Cu_1; costal fringe usually dark, but in a specimen pale brown at margin of cells R_1 and R_2 and before tip of R_1.



Brunettia ishiharai Tokunaga et Etsuko, sp. nov.
FIG. 27. Female, wing, hairs and scales omitted.
28. Female, lobes of sub-genital plate.

Head with eyes narrowly projected above and widely separated from each other as wide as five and a half facets. Maxillary palpi 4-segmented (6:16:16:20). Distal flagellar

segments broken off in all specimens. First antennal segment cylindrical; five flagellar segments, which are remained, fusiform and their relative lengths 30:24:24:24:24.

Wings (Fig. 27) 2.8 × 1.2 mm, comparatively broad, with tip round, membrane brown and entirely covered with slender scales, veins deep brown but distal ends of R_4, R_5, M_1 and M_2 pale brown. Venation: main veins radially arranged; base of R_{2+3} far beyond base of R_5, R_{2+3+4} as long as R_{2+3}, R_2 very long and about 7 times as long as R_{2+3}, RR about 0.14, bases of M_3 and M_4 separated, base of M_3 slightly beyond that of M_4, M_{1+2+3} a quarter as long as M_{1+2} (4:16), Cu_1 far separated from veins related to media and arised just beneath base of M_4, fork of M_{1+2} beyond that of R_{2+3}.

Two lobes of subgenital plate (Fig. 28) short and broad, narrowly separated by Y-shaped median incision and each lobe bruntly projected caudo-laterad.

Male.—Unknown.

Locality.—Shikoku, Japan.

Holotype.—Female mounted in Gater's solution; Mt. Saragamine, Ehime-ken; May 12, 1948.

Paratopotypes.—Females mounted in Gater's solution; May 12, 1948.

Type specimens.—Deposited in the entomological laboratory, Saikyo University; collected by Prof. Dr. T. Ishihara.

The present new species somewhat allied to *P. spinistoma* Tokunaga et Etsuko, however the two species may be distinctive by their specific structures of the female subgenital plates, the venation of wings and the coloration of the wings and legs. In the present species, the veins media are peculiar to us that the four branches are pectinately forked and the antennæ are also peculiar that there are no trace of ascoids on the flagellar segments remained. We are very much pleased to name this peculiar moth fly in relation to Prof. Dr. T. Ishihara who has kindly collected this species.

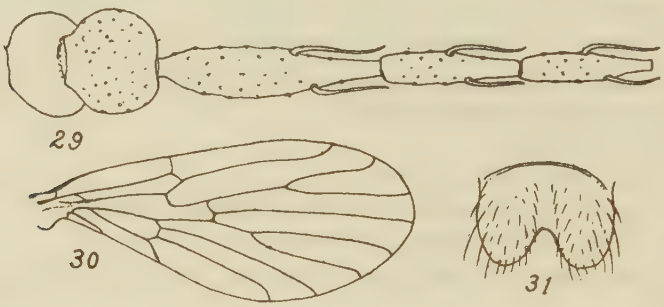
SYCORAX NIPPONICUS Tokunaga et Etsuko, sp. nov.

This species was collected at light along a mountain rapid stream.

Female.—Body length about 1.2 mm, not thickly haired, brown in alcoholic state but whitish gray in dry state.

Head oval, with eyes reniform in frontal aspect and widely separated from each other, frontal region without hairs, clypeus

entirely hairy. Maxillary palpi short, 4-segmented (11:12:-10:8); second segment with a group of spoonlike sensilla on ventral side; ultimate segment shortest and somewhat conical. Proboscis elongated conical, but far shorter than length of eye, and with small setigerous labella. Antennæ (Fig. 29) slender, filiform; basal segment subtriangular; second segment subspherical; first flagellar segment elongated fusiform and with a pair of simple ascoids beyond middle; second and following flagellar segments cylindrical, with an ascoid at middle and one beyond middle; relative length of basal eight segments about 7:8:28:18:18:17:17:16; other distal segments broken off.



Sycorax nipponicus Tokunaga et Etsuko, sp. nov.

FIG. 29. Female, basal five antennal segments, verticils omitted.
30. Female, wing, hairs omitted.
31. Female, lobes of subgenital plate.

Wings (Fig. 30) about 1.7 mm long, round on apical margin, not thickly hairy; veins yellowish brown. Venation: Sc, R_1, R_2, and R_3 curved up-wards at tip and median veins curved down-wards at tip, R_{2+3} far shorter than R_2 (20:26), RR about 0.77, distance between tips of R_1 and R_2 smaller than that between tips of R_2 and R_3 (12.5:14.5), R_{4+5} not forked, distal section of M_{1+2} about a half of R_{2+3} and a quarter of M_1 in length (10:20:40), distance between tips of M_1 and M_2 smaller than that between tips of M_2 and M_3 (11:12.5).

Subgenital plate (Fig. 31) broader than length, with two caudal lobes hemicircular and separated by a wide U-shaped caudal incision; cerci fleshy and angulated discoidal.

Male.—Unknown.

Locality.—Honshu, Japan.

Holotype.—Female mounted in Gater's solution; Osugi-tani, Mie-ken; June, 1952 (*M. Tokunaga*); deposited in the entomological laboratory, Saikyo University.

This species is closely allied to *S. silacea* Curtis, however in the allied European species, R_{2+3} is subequal to or only slightly longer than R_2 and distance between tips of M_1 and M_2 is larger than that between tips of M_2 and M_3, differing from the Japanese new species.

THE PHILIPPINE SPECIES OF PŒMENIINI (PIMPLINÆ, ICHNEUMONIDÆ, HYMENOPTERA)

By CLARE R. BALTAZAR[1]
Bureau of Plant Industry, Manila

TWO PLATES

The tribe Pœmeniini is represented in the Philippines by two genera: *Neoxorides* and *Eugalta*. Both genera may be distinguished from the other Philippine Pimplinæ by the presence of a scabrous or toothed area on the upper part of the temple. The genus *Neoxorides* is mainly Holarctic in distribution; it is now known to occur also in the Philippines, where it is represented by one new species with two subspecies. The genus *Eugalta* is apparently confined to the Orient. Several species have been described from Taiwan, India, Burma, and the Philippines.[2] The previously known species from the Philippines was described by Bingham under the name *Pimpla* (*Euxorides?*) *furcifer* in 1895. Five new species and two new subspecies are described in this paper.

The bulk of the materials studied are loan specimens from the United States National Museum. Other materials are in the collections of Drs. Henry and Marjorie Townes of North Carolina State College, and in the Bureau of Plant Industry at Manila. The types are in the Townes' collection and in the U. S. National Museum.

Key to the Philippine genera of Pœmeniini

1. First tergite short and broad at base, about 3.0 as long as basal width, punctate throughout; all tergites entirely punctate; mesoscutum not strongly trilobate, punctate throughout; hind leg with outer and inner tarsal claws gradually curved and with only one tooth; propodeum with sides almost at right angles with top; lower side of frons along orbits somewhat raised and with a protruberance; ovipositor sheath 1.6 to 1.8 as long as fore wing..... *Neoxorides.*

[1] The writer is indebted to Dr. Henry Townes for the help extended in locating references, synonymizing genera, and correcting the manuscript, and to Miss Luella Walkley for sending specimens and references pertaining to the genus.

[2] *Eugalta samoana* Fullaway was described from Samoa. Dr. Henry Townes studied the type and considers it a polysphinctine.

First tergite elongate and narrow at base, about 5.0 as long as basal width, rough basally and smooth apically; tergites mostly smooth, sometimes punctate only on the second and third tergites; mesoscutum strongly trilobate, impunctate or punctate with the median posterior part irregularly wrinkled and/or striate; hind leg with outer tarsal claw abruptly bent at right angles and with only one tooth, with an enlarged basal hair thickened at the apex, and inner tarsal claw gradually curved and bidentate; propodeum evenly convex; frons flat and without a protruberance at the side; ovipositor sheath 0.6 to 1.4 as long as fore wing..... *Eugalta.*

Genus NEOXORIDES Clément

Neoxorides CLÉMENT, Festschr. Embrik Strand 4 (1938) 517. Type: *Xorides nitens* Gravenhorst. Designated by Townes, 1951.

Aside from the distinguishing characters mentioned in the key to the genera, the Philippine species of *Neoxorides* differs from *Eugalta* in the following: Face somewhat wrinkled, with an upper median carinalike elevation; temple wide, about 0.3 to 0.5 length of eye, with the scabrous area occupying its upper 0.5; median lobe of mesoscutum not prominently separate from the lateral lobes; scutellum, metanotum, and tegula punctate; propodeum rugoso-punctate; areolet absent; tergites with punctures coarsest on the first, diameter of punctures gradually decreasing in size towards the last tergite; first sternite with a median blunt tooth; ovipositor very long, about twice as long as abdomen.

Similar to *Eugalta* in color markings. The antenna is banded with white in the only Philippine species but a specimen from Japan has the antenna entirely black.

NEOXORIDES LONGICAUDIS sp. nov.

Face with sparse long hairs and fine punctures; frons shiny and finely punctate on upper 0.5; sulcus present between posterior ocelli; vertex coarsely and closely punctate, the interspaces 0.5 to 1.5 diameter of punctures; temple widest on top, about 0.25 length of eye, its scabrous area slightly oblique to hind margin of eye and about 0.5 height of temple; antenna with 36 to 39 segments. Mesoscutum with very short inconspicuous hairs, closely and coarsely punctate throughout, the interspaces 0.5 to 1.0 diameter of punctures; scutellum and metanotum punctate, the interspaces 1.0 to 2.0 diameter of punctures; propodeum with a semicircular striate depression around petiolar attachment, with a broad shallow depression

behind spiracle; metapleurum with coarse, deep and somewhat elongate punctures, vertically wrinkled ventrally. Nervulus basad of the basal vein, first transverse cubital as long as second abscissa of cubital. First tergite coarsely and deeply punctate, the interspaces about 0.5 to 1.0 diameter of punctures, on its apical light transverse band with few scattered shallow punctures; second tergite coarsely, deeply and closely punctate except for apical margin, the punctures coarser basally which are about twice as wide as those apically; tergites closely and finely punctate; punctures decreasing in size towards the last tergite; first sternite rough with a median blunt tooth which is carinate across; ovipositor sheath 1.6 to 1.8 as long as fore wing.

Black with yellow to orange-brown markings. Frons, palpi, side of frons with the stripe continuing to vertex but not touching occipital carina, two triangular spots of mesoscutum, scutellum, metanotum, tegula, base of wings, pronotal collar, pleurum, subalar spot, and mesepimeron yellowish white; fore leg yellowish ventrally, dorsally black on coxa, brown on femur, tibia, and tarsus; middle leg yellowish ventrally except for a dark spot on apical 0.3 of coxa, dorsally yellowish on coxa and trochanters, femur yellowish brown, tibia brown, tarsus fuscous; hind leg with coxa yellow basally but apically black, first trochanter yellowish, second trochanter fuscous, femur orange-brown except for dark apical 0.3, tibia fuscous except for light basal 0.4, tarsus white except for dark apical 0.5 of last segment; all tergites with subequal yellow transverse band at apical margin.

Key to the subspecies of Neoxorides longicaudis

1. Propodeum with two apical whitish marks; mesopleurum rugoso-punctate, with a yellowish white longitudinal ventral spot in the female, but entirely black in the male; the two triangular spots on mesoscutum coalesced in the male, not coalesced in the female. Habitat: Luzon ..... *longicaudis longicaudis* subsp. nov.

Propodeum entirely black; mesopleurum punctate, with a yellowish subcircular antero-ventral spot in the female, but entirely black in the male; the two triangular spots on mesoscutum coalesced in both sexes. Habitat: Mindanao..... *longicaudis mindanensis* subsp. nov.

NEOXORIDES LONGICAUDIS LONGICAUDIS subsp. nov. **Plate 1, fig. 1.**

Female.—Fore wing 8.5 to 13.5 mm long. The distinguishing features are stated in the key to the subspecies.

Male.—Fore wing about 5.5 mm long. Similar to the female except for size and color differences as stated in the key.

Type.—♀, Mount Banahao, Laguna, Baker (U. S. National Museum No. 62498).

Paratypes.—♂, ♀, same data as type (♂, U. S. National Museum; ♀, Townes); 2 ♀, Mount Maquiling, Laguna, Baker (U. S. National Museum, and Bureau of Plant Industry at Manila).

NEOXORIDES LONGICAUDIS MINDANENSIS subsp. nov. **Plate 1, fig. 2.**

Female.—Fore wing 9.0 to 11.0 mm long. This subspecies can be distinguished from the other subspecies by the characters stated in the key.

Male.—Fore wing about 6.5 mm long. Very similar to the male of the other subspecies except for the difference in sculpture of the mesopleurum.

Type.—♀, Butuan, Agusan, Baker (U. S. National Museum No. 62499).

Paratypes.—♂, Butuan, Agusan; ♀, Kolambugan, Lanao, Baker (U. S. National Museum); ♀, Kolambugan, Lanao, Baker (Bureau of Plant Industry at Manila); ♀, Iligan, Lanao, Baker (Townes).

Genus EUGALTA Cameron

Eugalta CAMERON, Manchester Lit. Philos. Soc. Mem. 43 (1899) 135. Type: *Eugalta strigosa* Cameron. Designated by Ashmead, 1900.

Pseudeugalta ASHMEAD, U. S. Nat. Mus. Proc. 23 (1900) 55. Type: *Eugalta spinosa* Cameron. Monobasic.

Baliena CAMERON, Manchester Lit. Philos. Soc. Mem. 44 (1900) 101. Type: *Baliena leptopus* Cameron. Monobasic.

Tilgida CAMERON, Manchester Lit. Philos. Soc. Mem. 44 (1900) 108. Type: *Tilgida albitarsis* Cameron. Monobasic. New synonymy.

Bathymeris CAMERON, Ent. 39 (1906) 251. Type: *Bathymeris longipes* Cameron. Monobasic.

Formoxorides UCHIDA, Fac. Agr. Hokkaido Imp. Univ. Jour. 25 (1928) 14. Type: *Achorocephalus pilosus* Szepligeti. Original designation.

Eyes large, reaching to near base of the mandibles, slightly convergent below; clypeus more or less square, apically emarginate, not separated from the face by a suture but deeply impressed at base; mandible short and strong with one apical

tooth; face moderately convex; frons flat; temple narrow, about 0.15 to 0.25 length of eye, with a scabrous area occupying its upper 0.3 to 0.5; vertex wide and punctured; occipital carina distinct.

Mesoscutum strongly trilobate, its median lobe with elongate punctures; median posterior part of mesoscutum irregularly wrinkled and/or striate; propodeum elongate, with a gradual slope to the apex, irregularly wrinkled throughout, without carinæ; hairs on propodeum long; propodeal spiracle oval, placed at about the apical 0.4; side of pronotum smooth and shiny except for anterior area and posterior margin which are hairy and punctate; midsternal groove deep on pro- and mesosternum; tegula smooth and shiny; fore wing with or without an areolet, if present more or less triangular; legs elongate and slender; coxæ large and elongate; front tibia with a row of spines on inner side; basitarsi long and nearly as long as their tibiæ; fourth tarsal segment of all legs about 0.3 as long as the last tarsal segment; fore and middle legs with the tarsal claws bidentate; hind leg with outer tarsal claw abruptly bent at right angles and with only one tooth, with an enlarged basal hair thickened at the apex; inner tarsal claw gradually curved, bidentate with the inner tooth shorter.

First tergite, elongate, with long hairs, gradually widened towards apex, about 2.5 as long as its apical width, its spiracle near its anterior 0.4; tergites mostly smooth; gastrocœlus distinct; spiracles of tergites 2 to 7 small, almost round and placed before the middle of the segment; first sternite with or without a sharp ventral tooth; ovipositor long, as long as or longer than the abdomen.

The members of the genus *Eugalta* are medium to large species, measuring about 10 to 25 millimeters (excluding the ovipositor). The Philippine species are black with yellowish white to reddish-orange markings; antenna conspicuously banded with white; tergites marked with light transverse bands; hind basitarsus white; face and tegula (in all Philippine species studied) bright yellow, and scutellum in most species yellow. The wings are hyaline, faintly infuscate.

Key to the Philippine species of Eugalta

1. Areolet present; metapleurum with a large whitish spot except in *furcifera* 2
   Areolet absent; metapleurum entirely black 5

2. First sternite with a sharp subbasal ventral tooth; propodeum with an apical whitish semicircular mark; nervulus basad of the basal vein. Subspecies of *lunata* 3
   First sternite without a sharp ventral tooth; propodeum entirely black; nervulus interstitial with basal vein..... 4
3. Temple, frons, and median lobe of mesoscutum marked with white; posterior part of metasternum weakly wrinkled. Habitat: Mindanao.
   lunata lunata subsp. nov.
   Temple, frons, and median lobe of mesoscutum entirely black; posterior part of metasternum sharply wrinkled. Habitat: Samar and Biliran.
   lunata visaya subsp. nov.
4. Metapleurum and median lobe of mesoscutum entirely black; lateral lobe of mesoscutum with numerous coarse punctures.
   furcifera (Bingham)
   Metapleurum with a large whitish spot and median lobe of mesoscutum with a whitish V-shaped mark; lobe of mesoscutum impunctate.
   victoria sp. nov.
5. Scutellum yellow; first sternite with a median ventral tooth; mesoscutum and metapleurum marked with white; nervulus basad of the basal vein; ovipositor sheath 1.2 to 1.4 as long as fore wing.
   pulchra sp. nov.
   Scutellum black; first sternite without a ventral tooth; mesoscutum and metapleurum entirely black; nervulus interstitial with the basal vein; ovipositor sheath 0.6 to 0.8 as long as fore wing..... 6
6. Lateral lobe of mesoscutum impunctate; tergites 2 and 3 with very few or no coarse punctures; frons entirely black..... *bakeri* sp. nov.
   Lateral lobe of mesoscutum punctate; tergites 2 and 3 with numerous coarse punctures; frons with a whitish stripe along the orbits.
   philippina sp. nov.

EUGALTA FURCIFERA (Bingham). **Plate 1, fig. 3.**

Pimpla (*Euxorides?*) *furcifer* BINGHAM, Mag. Nat. Hist. Ann. 16 (1895) 445. Type: ♂, Cape Engaño, Luzon, Philippines (British Museum).

Calliclisis furcifer (Bingham) ASHMEAD, U. S. Nat. Mus. Proc. 28 (1904) 157.

Xorides furcifer (Bingham) ROMAN, Ark. för Zool. 15 8 (1913) 9.

Eugalta furcifera (Bingham) MORLEY, Fauna of British India, Hym. 3 (1913) 66.

Female.—Fore wing 10 to 13 mm long. Face with sparse long hairs and fine punctures, the interspaces about 2 to 3 times diameter of punctures; frons smooth and hairless on lower 0.5, hairy with very fine punctures on upper 0.5, with a median sulcus from above base of antenna to anterior ocellus and continuing between posterior ocelli; vertex hairy, coarsely punctate, the interspaces about 2 to 3 times diameter of punctures; clypeus slightly depressed above apical margin, hairy, with scattered punctures; temple longest at upper 0.3 (at the

region of scabrous area), about 0.15 length of eye, its scabrous area parallel to hind margin of eye and about 0.35 height of temple; antenna with about 39 sgements. Mesoscutum hairy; middle lobe of mesoscutum with close elongate punctures but anterior and posterior parts smooth and shiny; lateral lobe of mesoscutum with irregular coarse punctations, the interspaces about 1.5 to 2 times diameter of punctures; middle part of mesoscutum immediately behind median lobe irregularly wrinkled; scutellum hairy, its punctures smaller than those on mesoscutum, the interspaces about 2 to 3 times diameter of punctures; metanotum smooth and shiny; propodeum depressed just below spiracle; mesopleurum hairy, closely punctate with the interspaces 2 to 3 times diameter of punctures, and wrinkled ventrally; metapleurum of the same vestiture and sculpture as propodeum. Areolet present; nervulus interstitial with basal vein. All tergites smooth and shiny except for rough basal 0.4 of first tergite; first sternite rough and hairy in its central 0.4, without a sharp tooth; ovipositor sheath about 0.6 as long as fore wing.

Black with yellow to reddish-orange markings. Face, lower 0.8 of frons, palpi, ventral side of scape and pedicel, flagellar segments 8 to 17, pronotum, scutellum, metanotum in some specimens, tegula, subalar spot, mesepimeron, and propleural spot above fore coxa yellow to yellowish-white; fore leg yellow ventrally except for fuscous tarsus, dorsally brown to fuscous except for yellow trochanters; middle leg yellow ventrally except for fuscous trochanters and tarsi, dorsally fuscous to black with a small irregular dark spot at center of coxa; hind leg mostly dark, coxa yellow beneath with a broad dorsal spot which is notched at base, trochanters black, femur yellow on the outer side, tibia light on its basal 0.15 and apical tip, spurs light brown, basitarsus yellowish-white except for dark apical 0.1 to 0.2; first tergite with a basal band subequal in length to apical band; tergites 1 to 3 with subequal apical transverse yellow band, tergites 4 to 6 with apical transverse yellow band interrupted at center, tergite 7 almost entirely yellow except for its dark apical margin, tergite 8 dark except for its light apical tip; first sternite black except white base; the rest of the sternites brown.

The above color markings were observed in the larger specimens. The smaller specimens are darker and vary in color as follows: prosternum and front coxa entirely black; all legs

dark ventrally except for light markings on the underside of middle coxa at basal 0.3 and both ends of hind coxa; tergites 4 to 6 entirely black. A few specimens have the prosternum and front coxa entirely black, all legs more or less piceous, and tergites 4 to 6 entirely piceous.

Male.—Fore wing 10 to 12 mm long. Similar to the female in color and sculpture. Two small specimens have a broad median longitudinal furrow on the propodeum.

Specimens examined.—11 ♂, 7 ♀, Los Baños, Laguna, January, May, September, and October, 1953, Townes family (Townes). 14 ♂, 18 ♀, Mount Maquiling, Laguna, September, November, and December, 1953, January to May, 1954, C. R. Baltazar, R. Ballesteros, F. Candelaria, E. Dagang, and A. Marmeto; 4 ♂, 3 ♀, Montalban, Rizal, March and April, 1954, C. R. Baltazar, F. Candelaria, and M. Delfinado (all in the Bureau of Plant Industry at Manila). 31 ♂, 40 ♀, Mount Maquiling, Laguna; 9 ♂, 8 ♀, Los Baños, Laguna; 8 ♂, ♀, Mount Banahao, Laguna; ♂, Mount Limay, Bataan; ♂, Samar; ♂, Polillo Island; ♀, Biliran Island; ♀, Baguio, Mountain Province; 2 ♀, Iligan, Lanao; ♀, Butuan, Agusan; ♀, Kolambugan, Lanao, Baker; ♀, Lucban, Quezon, McGregor; ♀, Piñablanca, Cagayan, A. Duyag (all in the U. S. National Museum).

EUGALTA LUNATA sp. nov.

Face with very few hairs and scattered punctures, the interspaces about 3 to 4 times diameter of punctures; frons smooth, impunctate and with few scattered hairs; vertex with shallow punctations, the interspaces about 2 to 3 times diameter of punctures; a median groove arising from behind anterior ocellus and continuing to occipital carina; clypeus irregularly punctate, shallowly depressed at apical 0.5; temple widest below middle, about 0.15 as long as the eye, its scabrous area parallel to hind margin of eye and about 0.3 height of temple; antenna with about 39 segments. Mesoscutum with sparse hairs; median lobe of mesoscutum with few elongate punctures on anterior 0.5, behind smooth; lateral lobe of mesoscutum smooth and impunctate; median posterior part of mesoscutum with longitudinal striæ which are anteriorly divergent and posteriorly parallel; scutellum smooth and shiny with few scattered hairs; metanotum smooth and shiny; propodeum with apical semicircular white mark; a smooth, broad longitudinal depression from below the spiracle to the apical margin of propodeum;

mesopleurum hairy, its punctations shallow, the interspaces about 3 to 4 times diameter of punctures; metapleurum of similar vestiture as propodeum but with marked transverse wrinkling; posterior part of metasternum wrinkled. Areolet present; nervulus basad of the basal vein; ramellus distinct at the middle of the cubito-discoidal in some specimens. All tergites smooth and shiny except for rough basal 0.3 of first tergite; first sternite with a sharp median ventral tooth at its basal 0.3; ovipositor sheath about 0.65 as long as fore wing.

Black with an apical semicircular white mark of propodeum. Face, palpi, flagellar segments 8 to 15 and ventral side of 16 and 17, tegula, subalar spot, mesepimeron, and scutellum yellowish white.

There are two subspecies which differ markedly in the color of the frons, temple, mesoscutum, metanotum, and prosternum.

EUGALTA LUNATA LUNATA subsp. nov. **Plate 1, fig. 4.**

Female.—Fore wing 8.0 to 13.5 mm long. Black with yellow and orange markings. Side of frons, spot on top of eye, temple except for its black scabrous area and lower 0.2, ventral side of scape, of pedicel. and of first three flagellar segments, two longitudinal stripes of median lobe of mesoscutum which are basally convergent, central elevated area of metanotum, anterior part of side of pronotum, transverse spot with a median dorsal notch on lower 0.5 of mesopleurum, elongate subtriangular spot on top of metapleurum, and spot on propleurum (except in small specimens) yellowish white; fore leg almost entirely yellow ventrally except for brownish tarsal segments, dorsally brown except for trochanters; middle leg yellowish white ventrally except for brown tarsus, dorsally brown but very light brown on coxa and trochanters; hind leg tricolorous: coxa yellow with a longitudinal black band on the outside and a broad black spot on the inner basal 0.6, both spots merging at the base of the coxa, first trochanter entirely dark, second trochanter yellow, femur orange except for dark brown ends and dorsal surface, tibia brown except for yellowish streak on basal 0.6, spurs light brown, tarsus yellowish white except last two segments which are dark; tergites 1 to 8 end with a subequal apical transverse yellow band; first sternite entirely black, the rest of the sternites yellowish with a brown spot on the side of each sternite.

Male.—Fore wing 6.5 to 12.0 mm long. Similar to the female except that the light band on sixth and seventh tergites interrupted at center. In a few specimens temple entirely white, including its scabrous area.

Type.—♀, Parang, Cotabato, March 23, 1953, Henry Townes (Townes).

Paratypes.—♂, Pikit, Cotabato, June 13, 1953, Henry Townes (Townes); 33♂, 13♀, Butuan, Agusan; 7♂, 7♀, Kolambugan, Lanao; 2♂, 7♀, Iligan, Lanao; 7♂, 39♀, Surigao; 2♀, Dapitan, Zamboanga, Baker (U. S. National Museum No. 62483, Bureau of Plant Industry at Manila, and Townes).

EUGALTA LUNATA VISAYA sp. nov. **Plate 1, fig. 5.**

Female.—Fore wing 10.0 to 13.5 mm long. Black with yellowish to reddish markings. Differs from *E. lunata lunata* in having the frons, temple, mesoscutum, metanotum and propleurum entirely black; dorsal side of scape, pronotal collar reaching only the midline, posterior spot on mesopleurum, and posterior squarish spot on metapleurum yellowish white; fore leg yellowish white ventrally, dorsally coxa black, femur and tibia with a dark streak, tarsus dark; middle leg ventrally light except for dark tarsus, dorsally coxa reddish, trochanters yellowish white, femur, tibia, and tarsus fuscous; hind leg with coxa reddish except for two light spots on outer and inner sides, first trochanter black, second trochanter yellowish white, femur light externally but internally dark, tibia fuscous except for white basal 0.2, tarsus white except for last tarsal segment; tergites 1 to 3 with yellowish transverse apical band subequal, tergites 4 to 6 entirely black or the fourth with a thin band interrupted at center; tergite 7 mostly light dorsally.

Male.—Fore wing 8.0 to 13.0 mm long. Similar to the female except for sexual differences. Some specimens have the metanotum with a yellowish white spot.

Type.—♀, Samar, Baker (U. S. National Museum No. 62492).

Paratypes.—40♂, 82♀, Samar, Baker (U. S. National Museum, Bureau of Plant Industry at Manila, and Townes); 6♂, Biliran Island, Baker (U. S. National Museum).

EUGALTA VICTORIA sp. nov. **Plate 1, fig. 6.**

Female.—Fore wing 9.0 to 13.0 mm long. Face with sparse long hairs and scattered punctures, the interspaces about 4 to 5 times diameter of punctures; frons smooth and hairless on

lower 0.5, on upper 0.5 hairy and minutely punctured; vertex hairy, punctate, the interspaces about 2 to 3 times diameter of punctures; clypeus apically depressed with numerous long hairs; temple about 0.2 length of eye, its scabrous area parallel to hind margin of eye and about 0.35 height of temple; antenna with about 38 segments. Mesoscutum with long sparse hairs; middle lobe of mesoscutum with close elongate punctures on the anterior black area, smooth and shiny on the posterior yellow V-shaped mark; lateral lobe of mesoscutum almost entirely smooth except for widely scattered punctures and slight rugosity along anterior margin; central area of mesoscutum behind median lobe irregularly wrinkled; scutellum shiny with few scattered hairs and very fine punctures; metanotum smooth and shiny; propodeum with a longitudinal depression below spiracle to apex of propodeum; mesopleurum hairy and minutely punctate, the interspaces about 3 to 4 times diameter of punctures; metapleurum of the same vestiture and sculpture as propodeum. Areolet present; nervulus interstitial with basal vein. All tergites smooth and shiny except for rough basal 0.5 of first tergite; first sternite hairy and rough, without a sharp ventral tooth.

Black with yellow and reddish markings. Clypeus, ventral 0.75 of frons, palpi except tips, ventral side of scape, of pedicel, of first and second flagellar segments, flagellar segments 8 to 16 and 17 ventrally, pronotal collar reaching the midline, V-shaped mark on median lobe of mesoscutum, scutellum, central part of metanotum, tegula, base of wing, subalar spot, small anterior spot on mesopleurum, posterior elongate spot on mesopleurum above coxa, mesepimeron, elongate dorsal spot on metapleurum, and propleural spot above fore coxa yellowish white; fore leg yellowish white ventrally except for brown tarsus, dorsally yellowish brown except for yellow trochanters and black spot on coxa; middle leg yellow ventrally except for fuscous second trochanter and tarsus, dorsally brownish except for yellow coxa and first trochanter; hind leg with coxa yellow ventrally, dorsally with a broad black spot which is notched at base, its basal 0.2 yellow, trochanters black, femur orange-brown with a dark dorsal streak, tibia yellowish brown on its basal 0.2 and its apical 0.25, spurs brown, and tarsus piceous except for yellowish white basal 0.5 to 0.6 of basitarsus; tergites 1 to 6 with yellowish white apical transverse band; first tergite yellowish white on basal 0.4; tergite 7 with a broad

yellow transverse band; first sternite yellowish white on its basal 0.3; sternite 2 and basal 0.6 of sternite 3 yellowish white; the rest of the sternites black.

Male.—Fore wing 6.0 to 12.0 mm long. The color markings are rather variable. The larger males have color markings very similar to those stated for the female. In a few males the two spots on the mesopleurum are confluent. The smaller males tend to be darker. A group of specimens differ from the above as follows: prosternum entirely black; front coxa with brownish streak ventrally; middle and hind coxæ and hind femur entirely reddish brown; tergites 4 to 6 with apical band interrupted at center; hairs on face and hind coxa brown and white. The smallest male has the face blackish near clypeus; mesopleurum entirely black; tergites 4 to 6 entirely black and legs reddish to fuscous except for hind tarsus; hairs on face and hind coxa dark brown.

Type.—♀, Northwestern Panay, Baker (U. S. National Museum No. 62497).

Paratypes.—11 ♂, 12 ♀, same data as type (U. S. National Museum, Bureau of Plant Industry at Manila, and Townes); 3 ♂, ♀, Cuernos Mountains, Negros Oriental, Baker (U. S. National Museum); 12 ♂, Mount Canlaon, Negros Oriental, 3,600 feet, April and May, 1953, H., M., and D. Townes (Townes).

EUGALTA BAKERI sp. nov. **Plate 2, fig. 7.**

Female.—Fore wing 8.0 to 9.0 mm long. Similar to *furcifera* in having a median longitudinal sulcus on frons, nervulus interstitial with basal vein and the tergites smooth and shiny except for first tergite. Differs from *furcifera* in the following: areolet absent; lateral lobe of mesoscutum impunctate and shiny, posterior part of median lobe with 4 to 5 transverse striæ, mid-posterior area of mesoscutum with outcurving longitudinal striæ; propodeum evenly convex with metapleurum, without a depression below propodeal spiracle; ovipositor sheath about 0.8 as long as fore wing.

Similar to *furcifera* in color markings except for black frons and scutellum (yellow in *furcifera*).

Male.—Fore wing 9.0 mm long. Similar to the female except for sexual characters.

Type.—♀, Kolambugan, Lanao, Baker (U. S. National Museum No. 62494).

Paratypes.— ♂, ♀, same locality as type (♂, U. S. National Museum; ♀, Bureau of Plant Industry at Manila).

EUGALTA PHILIPPINA sp. nov. **Plate 2, fig. 8.**

Female.—Fore wing 7.0 to 11.0 mm long. Face with very few hairs and scattered punctures, the interspaces about 3 to 4 times diameter of punctures; frons smooth and hairless except for short hairs and fine punctures on the light stripe along orbitals; sulcus present between posterior ocelli; vertex punctate, the interspaces about 2 to 3 times diameter of punctures; temple widest on upper 0.4, about 0.2 length of eye, its scabrous area slightly oblique to hind margin of eye and about 0.45 height of temple; antenna with 36 to 38 segments. Mesoscutum almost hairless; middle lobe with very close elongate punctures; lateral lobe with coarse circular punctures, the interspaces about 1.0 to 1.5 diameter of punctures; middle part of mesoscutum immediately behind median lobe with anterior 0.5 transversely wrinkled and posterior 0.5 longitudinally wrinkled; scutellum and metanotum rugoso-punctate; propodeum evenly convex with metapleurum; mesopleurum rugoso-punctate except for smooth median transverse area, mesepimeron hairy and punctate; metapleurum of the same vestiture and sculpture as propodeum. Areolet absent; nervulus interstitial with basal vein; first transverse cubital 0.4 as long as second abscissa of cubital. First tergite rugoso-punctate except for apical transverse band; second tergite closely punctate, the interspaces about 0.5 to 1.0 diameter of punctures, apical transverse band smooth and shiny; third tergite closely punctate, with finer and shallower punctations than on second; tergite 4 to 8 impunctate; first sternite rough and longitudinally striate, without a tooth; ovipositor sheath about 0.75 as long as fore wing.

Black with yellow and orange markings. Face, palpi, side of frons, ventral side of scape, of pedicel, and of first three flagellar segments, flagellar segments 8 to 18, pronotal collar, tegula, base of wings, subalar spot, and mesepimeron yellowish white; fore leg yellowish white ventrally except for brown tarsus, dorsally brown except for yellow coxa and trochanters; middle leg yellowish white ventrally except for brown tarsus, yellow dorsally on basal 0.6 of coxa and trochanters, orange-brown femur and tibia, and fuscous on apical 0.4 of coxa and tarsus; hind leg tricolorous: coxa basally black, then yellow and apical 0.5 black, first trochanter yellow, second trochanter

fuscous, femur orange except for dark apex, tibia fuscous but banded basally and medially with white, tarsus fuscous except for white last segment; tergites 1 to 7 with subequal yellowish white transverse band on apical margin; first tergite with basal 0.2 yellowish; first sternite basally yellow and apically black.

Male.—Fore wing 7.0 to 11.0 mm long. Similar to the female except for the punctures on tergites 2 and 3 which are not as deep and conspicuous.

Type.—♀, Butuan, Agusan, Baker (U. S. National Museum No. 62495).

Paratypes.—♂, ♀, Kolambugan, Lanao; ♂, ♀, Iligan, Lanao; ♂, Los Baños; ♂, Mount Maquiling, Laguna; ♀, Samar, Baker (U. S. National Museum); ♂, Mount Maquiling, Laguna; ♀, Iligan, Lanao, Baker (Bureau of Plant Industry at Manila); ♂, Mount Maquiling, Laguna; ♀, Butuan, Agusan, Baker (Townes).

EUGALTA PULCHRA sp. nov. **Plate 2, fig. 9.**

Female.—Fore wing 8.5 to 14.0 mm long. Face with sparse long hairs and fine punctures; frons shiny and impunctate; vertex with irregularly scattered punctures, the punctures smaller behind ocelli and near eyes; clypeus with a semicircular hollow at center, impunctate above hollow, coarsely punctate at the sides and below hollow; temple widest at middle, about 0.25 as long as eye, its scabrous area slightly oblique to hind margin of eye and about 0.4 height of temple; antenna with about 41 segments. Mesoscutum with very short sparse hairs; middle lobe with close elongate punctures but anteriorly and laterally smooth and shiny; lateral lobe with large circular punctures, the interspaces about 0.5 to 1.0 diameter of punctures; middle part of mesoscutum immediately behind median lobe with 4 to 6 transverse striæ followed by longitudinally diverging striæ; scutellum shiny with few scattered hairs and numerous deep punctures; metanotum smooth and shiny; propodeum evenly convex with metapleurum; mesopleurum rugosopunctate and hairy except for upper hind triangular area, the interspaces about 1.0 to 1.5 diameter of punctures, mesepimeron shiny, almost impunctate, and with sparse hairs; metapleurum of the same vestiture and sculpture as propodeum. Areolet absent; nervulus basad of the basal vein; first transverse cubital 0.5 to 0.7 as long as second abscissa of cubitus. First tergite rough except apical transverse band; second tergite punctate,

the interspaces about 1.0 to 1.5 times diameter of punctures, punctures farther apart and coarser at base, apical transverse band smooth and shiny; third tergite with finer and shallower punctations than on second; tergite 4 to 8 impunctate, smooth and shiny; first sternite with a sharp tooth at about its middle; ovipositor sheath about 1.2 to 1.4 as long as fore wing.

Black with yellow and orange markings. Face, clypeus, palpi, side of frons extending up to vertex, temple except for its black scabrous area, postgena, ventral side of scape, of pedicel, and of the first three flagellar segments, flagellar segments 8 to 18, two longitudinal convergent stripes on median lobe of mesoscutum, two longitudinal parallel stripes at the center of mesoscutum, scutellum, central elevated area of metanotum, tegula, base of wings, anterior part of side of pronotum, transverse spot on lower 0.5 of mesopleurum, subalar spot, mesepimeron, and propleurum yellowish white; fore leg almost entirely yellowish white ventrally except for last four tarsal segments, dorsally brown except for yellow coxa, trochanters, and basal 0.2 of femur and a brown spot on outer apical end of coxa; middle leg yellowish white ventrally except for blackish apical 0.3 of coxa, second trochanter and tarsus, dorsally brownish except for yellow basal 0.6 of coxa and first trochanter; hind leg tricolorous: coxa whitish yellow with a black dorsal band slightly before the middle and its distal end black, first trochanter yellowish, second trochanter black, femur orange except for brown apical tip and a dorsal streak, tibia brown except for yellowish streaks on the outer basal 0.2 and apical 0.3, spurs and basitarsus yellowish white, and last four tarsal segments brown; tergites 1 to 7 with subequal yellowish white transverse bands on distal margin; first tergite with basal 0.25 yellowish; eighth tergite with yellowish distal margin; first sternite with basal 0.5 and its ventral tooth yellow, distal 0.5 black; second sternite entirely yellow; the rest of the sternites yellow centrally with longitudinal fuscous spots on sides.

Male.—Fore wing 7.0 to 10.0 mm long. Similar to the female except for the following differences in color markings: Yellow band of antenna on flagellar segments 9 to 19, segments 20 and 21 ventrally; last tarsal segment brown. Antenna with about 42 segments.

Type.—♀, Mount Canlaon, Negros Oriental, 3,600 feet, May 1, 1953, H., M., and D. Townes (Townes).

Paratypes.—2 ♂, same data as type, May 2 and 7, 1953, H., M., and D. Townes (Townes). ♂, ♀, Mount Maquiling, Laguna, February and March, 1954, C. R. Baltazar and A. Marmeto (Bureau of Plant Industry at Manila). ♀, Los Baños, ♂, 6 ♀, Mount Maquiling, and ♀, Mount Banahao, Laguna; 2 ♂, 2 ♀, Biliran Island; ♂, ♀, Cuernos Mountains, Negros Oriental; ♂, ♀, Northwestern Panay; 3 ♂, ♀, Iligan, Lanao; 8 ♂, ♀, Kolambugan, Bukidnon, Baker (U. S. National Museum Cat. No. 62496).

ILLUSTRATIONS

[Photographed by Dr. Henry Townes. Asterisk indicates that the specimen is the type.]

Plate 1

Fig. 1. *Neoxorides longicaudis longicaudis* subsp. nov.
2. *Neoxorides longicaudis mindanensis* * subsp. nov.
3. *Eugalta furcifera* (Bingham).
4. *Eugalta lunata lunata* subsp. nov.

Plate 2

Fig. 5. *Eugalta lunata visaya* * subsp. nov.
6. *Eugalta victoria* * subsp. nov.
7. *Eugalta bakeri* * sp. nov.
8. *Eugalta philippina* * sp. nov.
9. *Eugalta pulchra* * sp. nov.

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PLATE I.

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PLATE 2.

DERIVATION OF BOHR'S EQUATION ON THE ANGULAR MOMENTUM OF THE ELECTRON AND ITS RELATION TO MOSELEY'S LAW TO OBTAIN SEVERAL EXPRESSIONS OF RYDBERG'S CONSTANT AND THE RADIUS OF THE HYDROGEN ATOM [1]

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It is possible to derive Bohr's equation on the angular momentum of the electron and relate it to Moseley's law to obtain four expressions of Rydberg's constant (expressed in terms of frequency rather than wave number), and an expression for the radius of the hydrogen atom assuming that the orbits are arranged around the nucleus such that the first orbit is r/Z; the second $2r/Z$; the third $3r/Z$; and the *nth* orbit nr/Z, where Z is the atomic number and r the atomic radius. This is the same assumption made in a previous paper [2] although Moseley's law is treated differently.

Consider an electron of charge e at infinity being attracted by a nucleus of charge Ze where Z is the number of protons. The potential energy would be the work which has to be done on the system in bringing the electron from infinity up to a distance r from the nucleus. Thus

$$-\int_{\infty}^{r} Ze^2 \, da/a^2 = -Ze^2/r \qquad (1)$$

Let v = the velocity of the electron in the orbit of radius r. Since the force of attraction and the centrifugal force can be equated to each other, we have

$$mv^2/r = Ze^2/r^2 \qquad (2)$$

[1] Read before the American Association for the Advancement of Science, December 26, 1952, at St. Louis, Mo., U. S. A. under the title "Four Different Expressions for the Moseley's Law Constant."

[2] Balce, S. B. A note on Moseley's law. Philip. Jour. Sci. 65 (1938) 103; Chem. Abs. 32 (1938) 6937; British Chem. Abs. 1 (1938) 425; Chem. Zentr. § II 109 (1938) 3207.

where m is the mass of the electron.
Since the kinetic energy of the electron in the orbit is $mv^2/2$, equation (2) may be written as follows:

$$\text{Kinetic energy} = Ze^2/2r \qquad (3)$$

The total energy E of the system is made up of the kinetic energy $Ze^2/2r$ and the potential energy $-Ze^2/r$, hence

$$E = Ze^2/2r + (-Ze^2/r) = -Ze^2/2r \qquad (4)$$

It is seen that the potential energy is negative and equal to twice the total energy, i.e.,

$$\text{Kinetic energy} = -(\text{potential energy})/2 = -E = W \qquad (5)$$

where W is the total amount of energy needed to remove the electron to infinite distance.

Introducing Planck's equation (energy equals Planck's constant h times the frequency f) into equation (5), we get, together with equation (4)

$$hf = Ze^2/2r = mv^2/2 \qquad (6)$$

For an electron in the *nth* orbit of radius nr/Z, equation (6) becomes

$$hf_n = mv_n^2/2 = Z^2e^2/2nr \qquad (7)$$

Let us now assume that an electron falls into the *nth* orbit originally occupied by another electron, each revolving at the rate of N_n revolutions per second. The energy of each electron according to equation (7) is

$$hf_n = (1/2)\ m(\omega_n\ nr/Z)^2 = (1/2)\ m\ (2\pi N_n\ nr/Z)^2 \qquad (8)$$

Consider now both electrons with the characteristic frequency f_t, such that $f_t = 2f_n = N_n$. Equation (8) then becomes

$$hf_t = hN_n = 2(1/2)\ m\ (2\pi N_n\ nr/Z)^2 \qquad (9)$$

or

$$hN_n/2 = (1/2)\ m\ (2\pi N_n nr/Z)^2 \qquad (10)$$

The right-hand side of equation (10) represents the kinetic energy of one electron in the *nth* orbit. Simplifying equation (10) then substituting ω_n for $2\pi N_n$, we get

$$h = 2\pi n^2 r^2 m\omega_n/Z^2$$

or

$$mr^2\omega_n = hZ^2/2\pi n^2 \qquad (11)$$

For the hydrogen atom where Z and n both equal 1, equation (11) becomes

$$mr^2\omega_n = h/2\pi \qquad (12)$$

This is Bohr's postulate that the angular momentum of the electron in its orbit must be an integral value of $h/2\pi$. It should be observed that Bohr's equation which is usually written as $mr^2\omega_n = nh/2\pi$, contains n instead of n^2 as in equation (11).

Substituting $2\pi N_n$ for ω_n in equation (11) we get

$$mr^2\ (2\pi N_n) = hZ^2/2\pi n^2 \tag{13}$$

If we replace N_n with $2f_n$, since $f_n = N_n/2$, equation (13) becomes

$$mr^2\ (2\pi.2f_n) = hZ^2/2\pi n^2$$

or

$$f_n = hZ^2/8\pi^2 mr^2 n^2 \tag{14}$$

For a frequency difference $f = f_2 - f_1$, equation (14) gives

$$f_n = \frac{hZ^2}{8\pi^2 mr^2}\left(\frac{1}{n_2^2} - \frac{1}{n_1^2}\right) \tag{15}$$

Let us now consider Moseley's law for the frequency f_n of X-radiation emitted by atoms when bombarded with electrons,

$$f_n = BZ^2 \tag{16}$$

where f_n is the frequency of the emitted X-rays, B is a constant, and Z is the nuclear charge of the bombarded atoms. If f_n in equation (14) is substituted for f_n of Moseley's law, then an equation for the constant is easily obtained as follows:

$$B = h/8\pi^2 mn^2 r^2 \tag{17}$$

where we have considered only an atom of atomic number $Z = 1$. If we take only the electrons in the K orbit for which $n = 1$, then equation (17) becomes

$$B = h/8\pi^2 mr^2 \tag{18}$$

Substituting the following latest values [1] into equation (18)

$e = 4.8022 \times 10^{-10}$ abs. e.s.u. (electronic charge)
$h = 6.6238 \times 10^{-27}$ erg. sec. (Planck's constant)
$m = 9.1072 \times 10^{-28}$ gram (electron mass)
$r = 0.52943 \times 10^{-8}$ cm (radius of Bohr's orbit)

we get the value of $B = 3.28 \times 10^{15}$ which is Rydberg's constant expressed in terms of frequency instead of wave number.

[1] Pitzer, Quantum Chemistry. New York, Prentice Hall, Inc., 1953. P. 521.

From equation (7), $nr = Z^2e^2/2hf_n$ which when substituted in equation (14) we obtain the expression

$$f_n = hZ^2(2)^2h^2f_n^2/8\pi mZ^4e^4$$

or

$$f_n = 2m\pi^2Z^2e^4/h^3 \qquad (19)$$

Equating equations (16) and (19) we obtain the equation

$$B = 2m\pi^2e^4/h^3 \qquad (20)$$

Combining equations (18) and (20) we obtain the equation

$$r = h^2/4me^2\pi^2 \qquad (21)$$

and on substituting the necessary values mentioned above, we get for r the value 0.529×10^{-8} cm which is the radius of Bohr's orbit.

Equation (7) with equation (8) may also be written

$$(\tfrac{1}{2})m(2\pi N_n nr/Z)^2 = Z^2e^2/2nr \qquad (22)$$

If we now replace N_n by $2f_n$ and equate the resulting equation for f_n to equation (16), we obtain another equation for the value of B as follows:

$$B = e/4\pi m^{1/2}\ r^{3/2}\ (n = 1) \qquad (23)$$

Equating equation (7) to equation (16) gives another expression for B:

$$B = e^2/2nrh \qquad (24)$$

For $n = 1$, this expression becomes

$$B = e^2/2rh \qquad (25)$$

The different expressions for Rydberg's constant given in terms of frequency rather than wave number are as follows:

(1) $B = h/8mr^2\pi^2 = 3.28 \times 10^{15}$
(2) $B = 2m\pi^2e^4/h^3 = 3.28 \times 10^{15}$
(3) $B = e/4\pi m^{1/2}r^{3/2} = 3.28 \times 10^{15}$
(4) $B = e^2/2rh = 3.28 \times 10^{15}$

These values of r and of the Rydberg's constant are therefore found on the assumption that while emitting X-radiations there are two electrons on an orbit each having the same number of revolutions while the number of waves coming from each is one-half of the number of revolutions.

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[New names and new combinations are printed in *italics.*]

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